

ORIGINAL RESEARCH

The Value of Serum NR2 Antibody in Prediction of Post-Cardiopulmonary Resuscitation Survival

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Abstract

Introduction: N-methyl-D-aspartate receptor subunits antibody (NR2-ab) is a sensitive marker of ischemic brain damage in clinical circumstances, such as cerebrovascular accidents. We aimed to assess the value of serum NR2-ab in predicting the post-cardiopulmonary resuscitation (CPR) survival. **Methods:** In this cohort study, we examined serum NR2-ab levels 1 hour after the return of spontaneous circulation (ROSC) in 49 successfully resuscitated patients. Patients with traumatic or asphyxic arrests, prior neurological insults, or major medical illnesses were excluded. Participants were followed until death or hospital discharge. Demographic data, coronary artery disease risk factors, time before initiation of CPR, and CPR duration were documented. In addition, Glasgow coma scale (GCS), blood pressure, and survival status of patients were recorded at 1, 6, 24, and 72 hour(s) after ROSC. Descriptive analyses were performed, and the Cox proportional hazard model was applied to assess if NR2-ab level is an independent predictive factor of survival. **Results:** 49 successfully resuscitated patients were evaluated; 27 (55%) survived to hospital discharge, 4 (8.1%) were in vegetative state, 10 (20.4%) were physically disabled, and 13 (26.5%) were physically functional. Within 72 hours of ROSC all of the 12 NR2-ab positive patients died. In contrast, 31 (84%) of the NR2-ab negative patients survived. Sensitivity, specificity, positive and negative likelihood ratios of NR2-ab in prediction of survival were 54.5% (95%CI=32.7%-74.9%), 100% (95%CI=84.5%-100%), infinite, and 45.5% (95%CI=28.8%-71.8%), respectively. Subsequent analysis showed that both NR2-ab status and GCS were independent risk factors of death. **Conclusions:** A positive NR2-ab serum test 1 hour after ROSC correlated with lower 72-hour survival. Further studies are required to validate this finding and demonstrate the value of a quantitative NR2-ab assay and its optimal time of measurement.

Key words: NR2; outcome; cardiopulmonary resuscitation; survival

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Introduction:

Neurological recovery is the ultimate goal of successful cardiopulmonary resuscitation (CPR) (1). However, early post-CPR neurological assessment is a clinical challenge (1). Transient cerebral ischemia, sedative medications, and hypothermic resuscitation are common factors that interfere with early post-CPR neurological assessment (2, 3). Furthermore, there are insufficient data to support the reliability of advanced imaging techniques, including computed tomography (CT) and magnetic resonance imaging (MRI), for detection of early ischemic changes, even if they can feasibly be performed in unstable post-CPR patients (2, 4).

Recently, serum biomarkers have received growing attention for their ability to indicate neuronal ischemic damage. The greatest number of studies have been performed on S100 and Neuron Specific Enolase (NSE) (5-7), and both have failed to show a consistent correlation with post-CPR outcome in multiple studies. No biomarkers have been shown to be useful in the few hours after cardiopulmonary arrest (5). Furthermore, increase in serum levels of these biomarkers are not specific to brain tissue injuries, making them questionable as ideal biomarkers (6).

There is good evidence that the autoantibody toward N-methyl-D-aspartate receptor subunits (NR2) peptide can



serve as an early sensitive neurotoxicity biomarker in ischemic stroke patients (8). The NR2 peptide is a subcomponent of the N-methyl-D-aspartate receptor (NMDAR) and is ubiquitously distributed in the central nervous system (9-11). It has been shown that NMDAR is cleaved after blood-brain barrier rupture by means of different enzymes, including tissue plasminogen activator (t-PA), resulting in NR2B peptide release and prompting of an ultra-rapid antibody response (12, 13). NR2 antibody (NR2-ab) has been recognised as a neuronal ischemic biomarker. It is useful for differentiation of ischemic strokes from intra-cranial haemorrhages within the first few hours after the event. It also identifies a subgroup of patients with acute transient ischemic attacks (TIA) who are at a higher risk for clinical complications (13, 14). When measured in patients who underwent cardiopulmonary bypass for cardiac surgeries, serum NR2-ab was predictive of neurological complications (15). Additionally, data from animal models have shown increased levels of NR1, NR2A and, especially NR2B fragments in brain tissues after successful CPR has been performed on animals undergoing asphyxic cardiac arrest (16). Based on the above-mentioned points, we primarily raised the question of whether serum NR2-ab could predict poor outcome in successfully resuscitated patients.

Methods:

Study design and setting

This prospective diagnostic test study was conducted in the emergency departments of two teaching hospitals in Tehran, Iran, with the annual census around 45000 and 35000 patients, respectively. As a commitment to follow the under mentioned strict inclusion and exclusion criteria, nonprobability convenience sampling was used to recruit the cases in shifts covered by selected authors who served as attending physicians during November, 2011 to December, 2012. The protocol of this study was approved by Ethical Review Board of Iran University of Medical Sciences. Written consent was received from close family members of all participants. The code of ethics of the World Medical Association (Declaration of Helsinki, as revised in Seoul 2008) was fully read and followed in the present work.

Participants

Those successfully resuscitated to the point of return of spontaneous circulation (ROSC) were considered eligible for the study, if they maintained that condition for at least one uninterrupted hour. Patients with age < 18 years, traumatic or asphyxic arrest, head trauma within the past month, pregnant, or a history of disabling disorders, end stage diseases, loss of blood specimen, central nervous system diseases, persistent severe hypoxemia (O₂ saturation < 88%) for at least 10 minutes, or required additional CPR attempts within the first hour after ROSC were excluded.

Measurements

A 5 ml sample of non-heparinised blood was drawn from a peripheral vein access 1 hour after ROSC. We followed the scientific literature and manufacturer instructions for the semi-quantitative enzyme-linked immunosorbent assay (ELISA) kit for autoantibodies toward the NR2B fragment of the NMDA receptor (Human Glutamate (NMDA) Receptor Subunit Epsilon-2 Antibody (NR2B-ab) ELISA Kit, CUSABIO, China). Briefly, blood samples were centrifuged within 10 min of collection at 3000 cycles/min for 10 min, and the serum was moved and stored at -20 C° immediately. Thereafter, the samples were assessed using ELISA kits up to two months after the blood sampling date (13). An available microplate reader (Micro Reader 4 Plus, Hyperion, USA) was used to read the ELISA plates. Resuscitation efforts were started either by emergency medicine service personnel or immediately after arrival at emergency department by the emergency department staff, who had been trained to follow the 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science as the standard of care for cardiopulmonary arrest.

Data collection

Demographic data, medical history regarding hypertension (HTN), diabetes, and hyperlipidemia and smoking background were later collected from several sources, including the patients, if possible, close family members and available medical documents. Using a structured checklist, trained emergency medicine residents were responsible for gathering data on the time interval between cardiac arrest and CPR initiation, CPR duration, number of CPR attempts after the first hour of the ROSC, GCS, blood pressure, duration of hospitalization, survival outcome at 1, 6, 24, and 72 hours after ROSC, and neurological and functional outcome of the patients at the time of hospital discharge.

Statistical analysis

PASW 18.0 software (IBM SPSS Statistics, IBM Co., New York, United States) was used to perform all of the statistical analyses. Chi square and Mann-Whitney U tests were applied to compare the categorical and continuous data, respectively. Screening performance characteristics of NR2B-ab in prediction of 72-hour survival were calculated. All the reported values were two-sided. To account for the multiple testing effect in the comparison analyses of the baseline characteristics between the survived and non-survived groups, a $p < 0.01$ was considered significant. Using the Wald test, variables with significant statistical and clinical differences were selected for the Cox proportional hazards model, in which $p < 0.05$ was considered significant. The Mann-Whitney U test was employed to compare the GCS scores between the NR2 subgroups.



Table 1: Baseline characteristics of participants

Characters	Non-survivors n (%)	Survivors n (%)	P-value*
Number	22 (44.9)	27 (55.1)	
Male gender	10 (45.5)	14 (51.9)	0.66
Age (range)	56.5 (19-75)	53.0 (22-73)	0.94
Diabetes	7 (31.8)	5 (18.5)	0.28
Hypertension	9 (40.9)	11 (40.7)	0.99
Hyperlipidemia	10 (45.5)	5 (18.5)	0.04
Smoking	9 (40.9)	7 (25.9)	0.27
Arrest to CPR (minute)	10 (0-20)	10 (0-80)	0.35
CPR duration (minute)	32.5 (2-75)	20 (5-70)	0.18
Repeated CPR after 1 hour ROSC	10 (45.5)	4 (14.8)	0.02
Arrest to blood sampling (minute)	100 (62-150)	90 (65-210)	0.19
NR2-ab positive 1 hour after ROSC**	12 (54.5)	0 (0)	<0.001
GCS# score at 1 hour after ROSC	3 (3-5)	7 (3-15)	<0.001
MAP## at 1 hour after ROSC (mmHg)	69.2 (65-83)	80 (67-117)	<0.001

*Mann-Whitney U test and Chi square test were applied where appropriate, **ROSC: return of spontaneous circulation, # GCS: Glasgow coma scale, ##MAP: mean arterial pressure.

Results:

49 successfully resuscitated patients were evaluated; 27 (55%) survived to hospital discharge, 4 (8.1%) were in vegetative state, 10 (20.4) were physically disabled, and 13 (26.5%) were physically functional. The baseline characteristics of the patients are shown in Table 1. Sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios of NR2-ab in prediction of survival were 54.5% (95% CI=32.7-74.9), 100% (95% CI = 84.5-100), 100% (95% CI = 69.9-100), 73% (95% CI = 55.6-85.6), infinite, and 45.5% (95% CI=28.8-71.8), respectively.

Survival analyses

Within 72 hours of ROSC all of the 12 NR2-ab positive patients died. In contrast, 31 (84%) of the NR2-ab negative patients survived, and 27 of them survived to hospital discharge after 1 to 47 days hospitalization (Table 2). There were significant differences in mean arterial pressure (MAP) 1 hour after ROSC, NR2-ab, and GCS of survivors and non-survivors (Table 1). We omitted MAP in the Cox proportional hazards model because the 10 mmHg mean difference was not clinically significant. The subsequent analysis showed that both NR2-ab status and GCS were independent risk factors for death (Table 3). Additionally, we found a clinically and statistically significant difference between the GCS scores in the NR2 subgroups, at 1, 6 and 24 hours after ROSC (Table 4).

Discussion:

Based on the results of this study, there was a significant correlation between the positive results of serum NR2-ab 1 hour after ROSC and 72-hour survival of patients with successful CPR. Additionally, in the NR2-ab positive patients, GCS was significantly lower 1, 6, and 24 hours after ROSC, indicating a correlation between the bi-

omarker and negative outcome even before all the patients with positive tests had died. Our results are in agreement with a multitude of studies that focused on the association of this ischemic neuronal biomarker with the extent of neuronal damage and clinical outcome, particularly for ischemic stroke and TIA patients (8, 13, 17). We propose that the underlying pathophysiology of brain ischemia in cardiopulmonary arrest, which is global cerebral hypo perfusion, is similar to the local hypo perfusion observed in TIA and CVA patients. Thus, the mechanism of rise in the serum NR2 antibody level in post-CPR patients could be the same as that in TIA and CVA patients (16). In addition to the prognostic impact of NR2-ab, we observed that lower MAP and GCS 1 hour after ROSC also were indicative of poor survival outcome. The 10-mmHg difference between mean MAP values of the two groups was not considered clinically significant. Therefore, we disregarded the MAP values when generating the Cox proportional hazard model. We found that each was an independent prognostic factor for survival. Our data are in line with the idea of a more extensive approach toward factor inclusion in prognostic models. In our case, this meant the incorporation of both GCS score and serum biomarkers to create a more potent prognostic tool (18). Nevertheless, the widespread practice of sedating patients after resuscitation and the increasing application of therapeutic hypothermia in recent years affects the prognostic value of the GCS score (19), further highlighting the importance of alternative prognostic tools such as NR2-ab. An interesting finding of our study was the high specificity of serum NR2-ab level 1 hour after ROSC in prediction of survival. To the best of our knowledge, all other time-specific biomarker studies for post-CPR prognostication have used



Table 2: Cumulative survival rates for resuscitated patients based on serum NR2-ab (antibody) status 1 hour after successful resuscitation

Antibody	Outcome	Time, n (%)						Total
		6 hours	24 hours	72 hours	7 Days	14 Days	30 Days	
Negative								
	Died	2 (20)	6 (60)	6 (60)	9 (90)	10 (100)	---	10 (27)
	Survived	0 (0)	1 (4)	3 (11)	5 (19)	16 (60)	26 (96)	27 (73)
Positive								
	Died	1 (8)	8 (67)	12 (100)	---	---	---	12 (100)
	Survived	---	---	---	---	---	---	0 (0)

Table 3: Cox regression model of NR2 antibody status and Glasgow coma scale (GCS) 1 hour after return of spontaneous circulation

	Hazard Ratio (95% CI)	P-value*
NR2 antibody unadjusted	8.33 (3.02-23.01)	<0.001
NR2 antibody adjusted for GCS score	3.41 (1.25-9.25)	0.02
GCS score unadjusted	0.306 (0.11-0.84)	0.02
GCS score adjusted for NR2 antibody	0.35 (0.13-0.94)	0.04

*Wald test is applied.

Table 4: Comparison of Glasgow coma scale (GCS) in different times after successful resuscitation based on serum NR2 antibody status

GCS	NR2-ab positive*	NR2-ab negative*	Mann-Whitney U	P-value
1 hour after ROSC**	3 (3-3; 3-5)	5 (3-12; 3-15)	100.50	0.002
6 hour after ROSC	3 (3-3; 3-5)	8 (5-13; 3-15)	50.50	<0.001
24 hour after ROSC	3.00	11 (8-15; 3-15)	1.50	0.006
72 hour after ROSC	---	11.5 (9-15; 3-15)	---	---

*, Median (inter quartile range; range), **ROSC: Return of spontaneous circulation.

a longer time interval for blood sampling. The few studies on NR2-ab, which were conducted in other areas (e.g., TIA and CVA), chose longer intervals for blood sampling (a minimum of 3 hour after the event) (13). We postulate that the NR2 antibody level will increase in the few hours following an event. That means gaining more survival prediction sensitivity if sampling is repeated later. It remains to be determined whether later measurement of NR2-ab will have sufficient sensitivity to predict neurological outcome in survivors of cardiopulmonary arrest. The ultra-rapid antibody response to NR2 antigen remains a matter of debate. Because of the widespread expression of the NMDA receptor in the brain and the cerebral endothelial barrier, it is probable that antigenic sensitisation to the NMDA receptor occurs through minor neurological incidences, such as trivial head trauma over a person's lifetime. In this way, an anamnestic response to subsequent antigenic exposures, which occurs immediately after brain injury, can explain the rapidness of the antibody response. This hypothesis is in accordance with the observation that, in acute stroke patients, the serum NR2-ab level distinguishes between patients with or without prior brain ischemic events (17). Ubiquitous and exclusive expression of the NMDA receptor throughout the CNS results in spatial accuracy. Moreo-

ver, the rapid appearance of both NMDA receptor fragments and antibodies denotes temporal sensitivity for the NR2 peptide and antibody. Spatio-temporal properties of the NR2-ab may define it as an ideal biomarker in cases of ischemic neuronal injury (6, 20). Our finding, that a positive serum NR2-ab within 1 hour after ROSC was an independent prognostic factor of survival, supports this theoretical consideration. There are potential wider clinical applications for this test in the future. Carbon monoxide poisoning, shock, near-drowning and hypothermia are good examples. Those conditions have one thing in common: the extent of hypoxic neurologic damage is the main determinant of prognosis.

Limitations:

There were several limitations to our study. First, nonprobability convenience sampling and a relatively low number of patients were recruited, mainly because of highly selective inclusion criteria. Second, relying on a semi-quantitative kit to detect abnormal NR2-ab levels. Third, we did not measure the serum NR2-ab level serially. Indeed, 45% (N=10) of those who eventually died in their time at the hospital were NR2-ab negative. This suggests that those patients may have had positive test results if it had been repeated later. This is similar to the higher "cumulative sensitivity" that results from the se-



rial measurement of Troponin in cases of myocardial infarction (21). Fourth, because of funding limitations, we did not assess the NR2 peptide level simultaneously. It is unclear how this co-measurement could have improved the test characteristics. Fifth, we applied GCS as a measurement tool for neurological assessment. Although GCS was originally employed to predict outcome of patients with traumatic brain injury, it is of value to describe the level of consciousness in non-trauma patients. Specifically, it has been used to evaluate the outcome of successfully resuscitated patients (18).

Conclusion:

The status of serum NR2-ab level, 1 hour after ROSC, may be a predictor of short-term survival in successfully resuscitated patients. Further studies are required to demonstrate whether serial quantitative measurements of serum NR2-ab or a simultaneous measurement of the serum NR2 peptide provides additional prognostication value.

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Conflict of interest:

We declare that the authors of this article have no competing interests.

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Authors' contributions:

AB designed the study and participated in acquisition of data, interpretation of data, drafting manuscript, and revised manuscript for important intellectual content. SV participated in designing the study, acquisition of data, interpretation of data, and drafting manuscript. EM carried out statistical analysis and interpretation of data, drafted the manuscript, and revised manuscript for important intellectual content. SF participated in acquisition of data and drafting manuscript. ET participated in interpretation of data, and revising manuscript for important intellectual content. All authors read and approved the final manuscript, and agree to be accountable for all aspects of the work.

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