Prognostic Value of Venous Blood Ammonia in Patients With Out-of-Hospital Cardiac Arrest

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Background: Although studies have shown there is a correlation between increased blood ammonia level and hepatic encephalopathy, little information is available for patients with out-of-hospital cardiac arrest.

Methods and Results: We did a prospective study of 357 adult patients with out-of-hospital cardiac arrest whose venous blood ammonia levels were measured on arrival at the emergency room. The primary endpoint was favorable neurological outcome to hospital discharge. Of the 357 patients, 25 (7%) had a favorable neurological outcome. The venous ammonia levels were lower in the favorable neurological outcome group than in the unfavorable neurological outcome group (median, 50 μg/dl vs. 210 μg/dl, P < 0.0001). The adjusted odds ratio of ammonia levels for favorable neurological outcome was 0.98 (95% confidence interval, 0.96–0.99; P < 0.0001). The ammonia cutoff value of 93.0 μg/dl for the identification of favorable neurological outcome had the highest combined sensitivity and specificity, and higher ammonia levels were associated with more accurate negative predictive values (for ammonia levels of 192.5 μg/dl, the negative predictive value was 100%). Hyperammonemia was significantly related to patient variables that had a poor outcome (R = 0.439, P < 0.001). In addition, there was a significant correlation between venous ammonia level and arterial pH on emergency room arrival (R = 0.633, P < 0.001).

Conclusions: The measurement of ammonia was found to provide valuable information regarding neurological outcome to hospital discharge in adult patients with out-of-hospital cardiac arrest. (Circ J 2012; 76: 891–899)

Key Words: Ammonia; Cardiac arrest; Hypothermia; Neurological outcome
of cardiac arrest, or non-shockable rhythm. Excessive accumulation of ammonia induces neuronal metabolic derangement, promotes astrocyte swelling and perturbs cerebral nitric oxide metabolism, and there is a correlation between increased arterial blood ammonia concentration and hepatic encephalopathy. Some studies have demonstrated arterial blood ammonia increases in patients with cardiac arrest or shock, and that the ammonia level is associated with adverse conditions for resuscitation.

Therefore, we evaluated whether the blood ammonia level provided valuable information regarding neurological outcome for adult patients with out-of-hospital cardiac arrest. Our hypothesis was that hyperammonemia is associated with poor neurological outcome.

Methods

Patients

Between January 1, 2005 and December 31, 2008, patients who received ALS by paramedics after out-of-hospital cardiac arrest and who were subsequently transported to the emergency room (ER) were enrolled in this study. The inclusion criteria were age 18 years or older, and cardiac arrest on or after paramedic arrival at the patient’s side. The exclusion criteria were a tympanic-membrane temperature below 30°C on ER arrival, a lack of venous blood sample within 10 min of ER arrival, a past history of liver cirrhosis, or a do not attempt to resuscitate order. Patients were also excluded if their families who visited the ER after out-of-hospital cardiac arrest refused to give informed consent to allow the use of their ammonia data for this study.

Technical Information

Venous blood samples to measure the ammonia levels were taken within 10 min of ER arrival. The blood was immediately transferred into chilled disposable tubes containing an anticoagulant (heparin), and centrifuged at 3,500 g for 3 min. The plasma ammonia was then measured using a highly sensitive radioimmunoassay, ammonia-L (Serotetsuku Co Ltd; normal range 12–66 μg/dl, and the required interval of measurement of ammonia is ≈10 min). Our strategies of ALS and post-cardiac arrest care have been reported previously. Both paramedics and attending physicians performed CPR according to the international guidelines. Therapeutic hypothermia (34°C for 1–3 days) was induced within 3 h of ER arrival using intravenous ice-cold fluid and extracorporeal cooling methods. Then, in patients with suspected acute coronary syndrome, emergency coronary angiography was performed and percutaneous coronary intervention for coronary reperfusion therapy was done if a Thrombolysis in Myocardial Infarction grade of 0, 1 or 2 flow was observed in the relevant artery. In addition, extracorporeal CPR with emergency cardiopulmonary bypass plus intra-aortic balloon pumping for patients with out-of-hospital VF cardiac arrest was immediately performed when ROSC could not be achieved by conventional ALS within 10 min of ER arrival.

Resuscitation attempts were documented by both paramedics and attending physicians in accordance with a single data collection form. Individual data was collected according to the Utstein Style reporting guideline, inclusive of information of past history from the family and/or the medical record before cardiac arrest, and treatment and clinical findings after ER arrival.

Study Endpoints

The primary endpoint was favorable neurological outcome at the time of hospital discharge, defined according to the Glasgow-Pittsburgh cerebral performance category of 1 (good performance) or 2 (moderate disability); the other categories were 3 (severe disability), 4 (a vegetative state), and 5 (death). The neurological outcome was assessed by physicians without any knowledge of the study. The secondary endpoint was ROSC, which was indispensable for neurologically intact survival from cardiac arrest, defined as spontaneous palpable pulse of the carotid artery for longer than 20 min.

Statistical Analysis

The patients were divided into 2 groups according to neuro-
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...logical outcome at the time of hospital discharge (favorable neurological outcome group vs. unfavorable neurological outcome group). The mean values and proportions of the baseline variables were compared between the 2 groups using the Mann-Whitney U test for continuous variables, and the chi-square test for categorical variables, as appropriate. The Mann-Whitney U test was used to evaluate the association between ammonia levels and the study endpoints. The multiple logistic regression analysis was done for independent predictors of the primary endpoint, including age, sex, cause of cardiac arrest, witnessed cardiac arrest, bystander-initiated CPR, initial recorded cardiac arrest rhythm, resuscitation-related time intervals, ammonia level and therapeutic hypothermia. We constructed a receiver-operating characteristic (ROC) curve to illustrate the various cutoff values of ammonia to differentiate favorable and unfavorable neurological outcomes. A stepwise multiple regression analysis was used to evaluate the correlation between ammonia levels and prehospital prognostic-related factors, including age, cause of cardiac arrest, witnessed cardiac arrest, bystander-initiated CPR, initial recorded cardiac arrest rhythm, resuscitation-related time intervals and ROSC on ER arrival. Finally, in patients whose arterial blood gas was measured within 10 min of ER arrival, we evaluated the correlation between the venous ammonia level and pH using a polynomial regression model, and analyzed the area under the ROC curve of the arterial blood gas (PaCO₂, bicarbonate) to differentiate the primary endpoint.

All analyses were performed using the SPSS software package (version 16.0J SPSS, Chicago, IL, USA).

**Results**

During the study period, a total of 550 patients who suffered out-of-hospital cardiac arrests were transported to the ER. Of these, 193 were excluded, so 357 adult patients with out-of-hospital cardiac arrest were eligible for enrollment in the study (Figure 1). The time interval between call receipt and venous blood samples to measure the venous ammonia levels ranged from 19 to 72 min, with a mean (± SD) of 38±9 min, a median of 37 min, and 25th and 75th percentile values of 32 and 43 min.
Figure 2. Analyses of the 357 adult patients who suffered out-of-hospital cardiac arrest. (A) Ammonia levels in the ROSC group vs. the Non-ROSC group. (B) Ammonia levels between the favorable neurological outcome group vs. the unfavorable neurological outcome group. (Each box presenting ammonia level shows the median and interquartile range, and each bar 25th and 75th percentiles.) ROSC, return of spontaneous circulation.

Figure 3. Adjusted odds ratios for neurological outcome to hospital discharge associated with selected factors in 357 adult patients with out-of-hospital cardiac arrest. CI, confidence interval; CPR, cardiopulmonary resuscitation; VF, ventricular fibrillation; VT, ventricular tachycardia.
respectively. The ammonia levels ranged from 10 to 400 μg/dl, with a mean (±SD) of 211±132 μg/dl, a median of 196 μg/dl, and 25th and 75th percentile values of 84 and 341 μg/dl, respectively.

At baseline, significant differences were seen between the favorable neurological outcome group and the unfavorable neurological outcome group in age, sex, cause of cardiac arrest, location of cardiac arrest, proportion of witnessed cardiac arrests and bystander-initiated CPR, initial recorded cardiac arrest rhythm, the treatments of ALS, post-cardiac arrest care, some resuscitation-related time intervals and the time interval between call receipt and venous blood sample to measure the ammonia (Table).

Of the 357 patients, 119 (33.3%) achieved ROSC, including 84 (23.5%) by conventional CPR and 35 (9.8%) by extracorporeal CPR. The ammonia level was lower in these patients than in patients who did not achieve ROSC (median, 117 μg/dl vs. 235 μg/dl, P<0.0001) (Figure 2A). In total, 25 (7.0%) of the 357 study patients had a favorable neurological outcome at hospital discharge. The ammonia levels were lower in these patients than in patients with unfavorable neurological outcomes (median, 50 μg/dl vs. 210 μg/dl, P<0.0001) (Figure 2B).

In the multiple logistic regression analysis for favorable neurological outcome in all study patients, ammonia levels
were an independent predictor, with an adjusted odds ratio of 0.976 (95% confidence interval (CI), 0.964–0.988; P<0.0001) (Figure 3). Other independent predictors of a favorable neurological outcome were the call-to-patient’s-side time interval and therapeutic hypothermia. When the location of cardiac arrest was included in the analysis, the results did not change. In addition, in the multiple logistic regression analysis for favorable neurological outcome in the subgroup of patients with ROSC, the result of the ammonia levels did not change. The capacity of ammonia to differentiate favorable neurological outcome from unfavorable neurological outcome was assessed with a ROC curve analysis (Figure 4). The area under the ROC curve was 0.88 (95%CI, 0.83–0.92; P<0.001). The ammonia cutoff value of 93.0 μg/dl for the identification of a favorable neurological outcome had the highest combined sensitivity and specificity, with an accuracy of 79%. Higher ammonia levels were associated with more accurate negative predictive values (for an ammonia level of 192.5 μg/dl, the negative predictive value was 100%). Stepwise logistic regression analysis showed that ammonia levels correlated with the prognostic-related factors of witnessed arrest, shockable rhythm as an initial recorded cardiac arrest rhythm and ROSC on ER arrival (the estimated ammonia level in venous blood on ER arrival=268–70×presence of witnessed arrest−36×presence of shockable rhythm as an initial recorded cardiac arrest rhythm−93×presence of ROSC on ER arrival, R=0.422, P<0.0001). In the 289 patients whose arterial blood gas was measured within 10min of ER arrival, there was a significant correlation between the ammonia level and pH (R=0.633, P<0.001) (Figure 5).

Discussion

Hyperammonemia on ER arrival was associated with poor achievement of ROSC and poor frequency of a favorable neurological outcome at the time of hospital discharge (Figures 2,3). No patients had a favorable neurological outcome at the time of hospital discharge when the venous ammonia level on ER arrival increased to ≥192.5 μg/dl (Figure 4).

Several mechanisms might account for hyperammonemia.11–13 Ammonia metabolism primarily involves 5 organs: the gut, kidneys, muscle, brain and liver. The causes of hyperammonemia can be divided into processes that increase ammonia production or decrease ammonia elimination. Several studies have reported that hemorrhagic shock increases ammonia in the blood both by increased ammonia production in the gut, kidney, muscle, etc. and by decreased ammonia elimination in the liver, kidneys and skeletal muscle, which leads to cellular dysoxia.11–13 It is impossible to maintain regional organ and tissue perfusion, especially splanchnic perfusion, during cardiac arrest and the early phase after ROSC.1,2,7 In patients with out-of-hospital cardiac arrest, Ishida et al demonstrated a correlation between the arterial ammonia level and the arterial lactic acid level at the time of ER arrival, and speculated that ammonia was released from the red blood cells as a response to acidosis during cardiac arrest.14 Regarding the blood, Barta and Babusikova showed that the ammonia level increases significantly in stored blood and stored plasma, and that the speed of increase in the ammonia level in the stored blood was approximately 3-fold as fast as that in the stored plasma.15 Yanagawa et al also demonstrated that the arte-

\[ \text{Ammonia (μg/dl)} = 268 - 70 \times \text{presence of witnessed arrest} - 36 \times \text{presence of shockable rhythm as an initial recorded cardiac arrest rhythm} - 93 \times \text{presence of ROSC on ER arrival}, R=0.422, P<0.0001 \]

\[ \text{Ammonia cutoff value} = 93.0 \mu g/dl \]
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rial ammonia level had a relationship with pH in the arterial blood.\textsuperscript{14} Nagamine demonstrated that the arterial ammonia level on ER arrival could be used as a reference to estimate the time of cardiac arrest (ie, duration of cardiac arrest).\textsuperscript{15} In the present study, the area under the ROC curve of the venous ammonia level to differentiate the primary endpoint was larger than that of the bicarbonate level and the PaCO\textsubscript{2} level in the arterial blood (Figure 4). Finally, there was a significant correlation between the venous ammonia level and the pH in the arterial blood on ER arrival (Figure 5). The ammonia levels were lower in these patients than in patients with bystander-initiated CPR (median, 106 μg/dl vs. 251 μg/dl, P<0.0001), ven- tricular tachycardia, pulseless ventricular tachycardia as initial cardiac rhythm (median, 125 μg/dl vs. 218 μg/dl, P<0.0001), and failure of ROSC by Emergency Medical Service respondents (median, 70 μg/dl vs. 208 μg/dl, P<0.0001) (Figures 6A–C). On the basis of these findings, it is suggested that venous ammonia levels during cardiac arrest and the early phase after ROSC are associated with the degree of regional organ and tissue hypoperfusion, and speculated that ammonia was released from the red blood cells as a response to acidosis during cardiac arrest.

Although there are a few studies of the ammonia levels in arterial blood for patients with out-of-hospital cardiac arrest,\textsuperscript{14–16} we measured the venous blood ammonia because less technical skill is required to take venous blood samples in the resuscitation phase. In fact, approximately half of the study patients had no measurement of arterial blood gas within 10 min of ER arrival.

Many factors during the resuscitation process, inclusive of post resuscitation care, have been associated with neurological outcome.\textsuperscript{1,2,7–8} A maximum end-tidal carbon dioxide of 10 mmHg, as a marker of cardiac arrest during CPR, is associ- ated with adverse outcomes.\textsuperscript{5,4} Several chemicals are released from the brain into the blood and cerebrospinal fluid after cardiac arrest. Of those, the serum concentration of neuron-specific enolase (NSE) has appeared promising as a predictor of poor neurological outcome.\textsuperscript{25,27} Elevated levels of S100 (glial protein), the BB fraction of creatine kinase in cerebrospinal fluid or serum, and neurofilament protein have revealed a specificity for poor neurological outcome.\textsuperscript{28} The biochemical markers of NSE and S100 at 24–72 h after ROSC have been shown to provide useful predictive neurological information, but these markers have not been used to predict neurological outcome during cardiac arrest or immediately after ROSC.\textsuperscript{28} In this study, the venous ammonia level was measured within 1 h of cardiac arrest, and hyperammonemia was associated with poor neurological outcome. These findings suggest that early measurement of the venous blood ammonia level is use- ful for predicting neurological outcome in adult patients with out-of-hospital cardiac arrest.

Ammonia is generally considered a key factor in hepatic encephalopathy pathogenesis, with astrocytes being the principal target of ammonia neurotoxicity.\textsuperscript{11,12} When ammonia levels rise acutely within the brain, astrocyte and neuron functions are affected. Astrocytes rapidly metabolize ammonia to glutamine, but the subsequent rise in intracellular osmolality causes astrocyte swelling and loss.\textsuperscript{29,30} Inflammatory cytokines are released by astrocytes.\textsuperscript{30,4} Ongoing oxidative and nitrosta- tive stress cause additional astrocyte loss through apoptosis.\textsuperscript{29}

In the remaining astrocytes, ammonia-mediated inhibition of alpha-ketoglutarate dehydrogenase and the depletion of carboxylic acids for glutamine synthesis paralyses the Krebs cycle.\textsuperscript{31,32} Adenosine 5′-triphosphate and nicotinamide adenine dinucleotide production falls, which hinders the metabo-

lism of pyruvate to lactate. Lactate levels in the astrocytes and brain increase. Decreased expression of glutamate receptors in the astrocytes causes increased concentration of glutamate, and seizures may result. Effective cerebral autoregulation is lost and cerebral edema and intracranial hypertension may develop. In addition, cerebral herniation has been shown to occur when the arterial ammonia level is >146 μg/dl in patients with acute liver failure.\textsuperscript{33} In the present study, no patient had a favorable neurological outcome when the venous ammonia level on ER arrival was ≥192.5 μg/dl. These findings suggest that hyperammonemia causes poor neurological outcome in patients with out-of-hospital cardiac arrest.

Study Limitations

There are several limitations to our study. First, it was not a multicenter study for resuscitation after out-of-hospital cardiac arrest. Second, favorable neurological outcome at the time of hospital discharge was an insufficient outcome in patients who were moved to an extended care facility etc. However, the results of this study did not change when neurological outcome was assessed at 30 days after cardiac arrest. The 312 patients was CPC of 5 (death) at the time of 30 days after cardiac arrest. Third, this study was conducted to search for prognostic factors during the resuscitation process, inclusive of in-duction of therapeutic hypothermia, but there were a small number of patients who had a measurement of venous ammonia before induction of hypothermia (within 10 min of ER ar- rival). Although we performed therapeutic hypothermia in 96 of the patients with out-of-hospital cardiac arrest, 36% (35/96) of the patients did not have a measurement of venous ammonia before induction of cooling. However, a favorable neurological outcome occurred in 74% (20/27) of patients treated with hy- pothermia after ROSC by conventional CPR, excluding pa- tients who attained ROSC through emergency cardiopulmo- nary bypass. The evidence strongly supports mild therapeutic hypothermia as an effective therapy for post-cardiac arrest syndrome.\textsuperscript{1,2,7,31–33} Both animal and our previous studies have demonstrated a benefit of very early cooling.\textsuperscript{37–39} We per- formed a multicenter study of therapeutic hypothermia in comatose survivors after out-of-hospital cardiac arrest (J-PULSE hypothermia; NCT00901134), and concluded that the venous blood ammonia level on ER arrival could be one of the criteria for induction of therapeutic hypothermia after cardiac arrest.\textsuperscript{40}

In addition, some studies have reported that hypothermia abrogates many of the metabolic effects of ammonia for pa- tients with hepatic encephalopathy.\textsuperscript{41} Fourth, we only measured the ammonia concentration once, at the time of ER arrival. If the ammonia was measured at the time of paramedic arrival at the patient’s side or at the time of achievement of ROSC, the cutoff points and upper limit of ammonia level for neurological outcome might have been slightly different.

Conclusion

The measurement of ammonia in venous blood on ER arrival was found to provide valuable information regarding neuro- logical outcome in adult patients with out-of-hospital cardiac arrest.

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