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Early Activation of the Kynurenine Pathway Predicts Early Death and Long-term Outcome in Patients Resuscitated From Out-of-Hospital Cardiac Arrest

Giuseppe Ristagno, MD, PhD; Roberto Latini, MD; Jukka Vaahersalo, MD; Serge Masson, PhD; Jouni Kurola, MD, PhD; Tero Varpula, MD, PhD; Jacopo Lucchetti, MBiol; Claudia Fracasso, MBiol; Giovanna Guiso, MBiol; Alessandro Montanelli, MD; Simona Barlera, MSc; Marco Gobbi, PhD; Marjaana Tiainen, MD, PhD; Ville Pettilä, MD, PhD; Markus B. Skrifvars, MD, PhD, EDIC, FCICM; for the FINNRESUSCI Investigators*

Background—The kynurenine pathway (KP) is the major route of tryptophan (TRP) catabolism and is activated by inflammation and after cardiac arrest in animals. We hypothesized that the KP activation level correlates with severity of post–cardiac arrest shock, early death, and long-term outcome.

Methods and Results—Plasma was obtained from 245 patients enrolled in a prospective multicenter observational study in 21 intensive care units in Finland. Time to return of spontaneous circulation, lowest systolic arterial pressure, and bicarbonate during the first 24 hours were collected. A cerebral performance category of 3 to 5 defined 12-month poor outcome. Plasma TRP and KP metabolites, kynurenine (KYN), kynurenic acid, 3-hydroxyanthranilic acid, and the ratio of KYN to TRP were measured by liquid chromatography and mass spectrometry. All KP metabolites at intensive care unit admission were significantly higher in cardiac arrest patients with a nonshockable rhythm compared to those with a shockable rhythm, and kynurenic acid and 3-hydroxyanthranilic acid correlated with time to return of spontaneous circulation. Patients with higher levels of KYN, KYN to TRP, kynurenic acid, and 3-hydroxyanthranilic acid had lower 24-hour systolic arterial pressure and bicarbonate. All KP metabolites and the ratio of KYN to TRP, but not TRP, were significantly higher in patients who died in the intensive care unit in comparison to those who survived. Multivariable logistic regression showed that high kynurenic acid (odds ratio: 1.004; 95% confidence interval: 1.001 to 1.008; $P=0.014$), and 3-hydroxyanthranilic acid (odds ratio: 1.011; 95% confidence interval: 1.001 to 1.022; $P=0.03$) were independently associated with 12-month poor outcome and significantly improved risk reclassification.

Conclusions—KP is activated early after cardiac arrest and is associated with severity of post–cardiac arrest shock, early death, and poor long-term outcome. (*J Am Heart Assoc.* 2014;3:e001094 doi: 10.1161/JAHA.114.001094)

Key Words: brain • cardiac arrest • kynurenine • shock • survival • tryptophan

From the IRCCS – Istituto di Ricerche Farmacologiche “Mario Negri,” Milan, Italy (G.R., R.L., S.M., J.L., C.F., G.G., S.B., M.G.); Department of Anesthesiology and Intensive Care Medicine, Helsinki University Central Hospital, Helsinki, Finland (J.V., T.V., V.P., M.B.S.); Department of Intensive Care, Kuopio University Central Hospital, Kuopio, Finland (J.K.); Clinical Investigations Laboratory, Humanitas Clinical and Research Center, Milan, Italy (A.M.); Department of Neurology, Helsinki University Hospital, Helsinki, Finland (M.T.).

*A full list of the FINNRESUSCI Investigators can be found in the appendix.

Correspondence to: Markus B. Skrifvars, MD, PhD, EDIC, FCICM, Department of Anesthesiology and Intensive Care Medicine, Helsinki University Central Hospital, Topeliuksenkatu 5, PL 266, 00029 HUS Helsinki, Finland. E-mail. markus.skrifvars@hus.fi

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Despite initially successful resuscitation, morbidity and mortality following cardiac arrest (CA) remain high.¹ The pathophysiological state recently named “post–cardiac arrest syndrome” is characterized by myocardial dysfunction with circulatory shock, systemic inflammation with activation of the clotting system, and evolving brain injury.^{2,3} Accordingly, there are evident similarities between sepsis, septic shock, and post-CA syndrome.⁴ On reperfusion following CA, a possible sequence of events includes a systemic inflammatory response that contributes to worsening of circulatory shock and neurological injury.^{2,4}

Recently, the activation of the enzyme indoleamine 2,3-dioxygenase, which is responsible for the metabolism of the essential amino acid tryptophan (TRP) to kynurenine (KYN)—the first step of the so-called kynurenine pathway (KP)—has been highlighted in septic shock patients.⁵ The activation of this pathway results in increased levels of KYN, which in turn

cause vasodilatation.⁵ Interestingly, KP activation has also been implicated in instances accounting for neurological injury (ie, stroke and cerebral dysfunction) in intensive care unit (ICU) patients and in adverse prognosis in patients with coronary artery disease.^{6–8} Downstream metabolites of KP include kynurenic acid (KYNA) and 3-hydroxyanthranilic acid (3-HAA)^{9–11}; the former has neuroprotective properties, whereas the latter has been associated with neurological damage and apoptosis of neurons.^{11,12} Thus, these 2 KP metabolites have been implicated in the pathogenesis of evolving neurological injury after an ischemic insult.⁶

In a study including both small and large animal models of CA and cardiopulmonary resuscitation, we recently demonstrated the early activation of the KP after successful resuscitation.¹³ In the present observational cohort study, we examined the KP in a large population of patients resuscitated from out-of-hospital CA. We hypothesized that the KP is activated after CA and that high levels of activation at ICU admission are associated with severity of post-CA hypotension, early death, and poor 12-month neurological outcome.

Methods

Patients

Patients included in the present study were part of the Epidemiology and Cost-effectiveness of Out-Of-Hospital Cardiac Arrest in Finland (FINNRESUSCI) study, which was a prospective observational cohort study conducted at 21 hospitals in Finland between March 1, 2010, and February 28, 2011.¹⁴ The study was approved by the ethics committee of each participating hospital. Informed consent from the patients' next of kin was obtained for data collection and blood sampling. For this study, we included patients with both shockable and nonshockable initial rhythms in whom blood samples were obtained at ICU admission. Blood was also drawn from 10 healthy volunteers matched for age and sex with the study population.

Blood Samples

Blood samples were collected into EDTA tubes and centrifuged, and plasma was stored at -70°C . At analysis, samples were thawed and divided into aliquots. Plasma levels of TRP and its metabolites, KYN, KYNA, and 3-HAA, were measured blinded to case identity using high-performance liquid chromatography coupled with mass spectrometry. We used blood samples from healthy subjects to serve as controls. Briefly, human plasma samples (100 μL) were mixed with 10 μL of internal standard (5-HTRP, 1 ng/ μL or deuterated metabolites [TRP-d5, 12 ng/ μL ; KYNA-d4, 0.4 ng/ μL ; KYNA-d5,

0.012 ng/ μL ; 3-HAA-d2, 0.012 ng/ μL], final concentrations) that was deproteinized by adding 400 μL of cold methanol and incubated for 1 hour at -20°C . After centrifugation for 10 minutes at 14 000g, the supernatants were collected and centrifuged again. Supernatants were dried under nitrogen flow; residues were dissolved in 100 μL of 1% acetonitrile in 0.1% formic acid and transferred to an autosampler vial insert, and 40 μL of supernatant was injected directly in the high-performance liquid chromatography system (Alliance separations module 2695; Waters Corp). The chromatographic separation was obtained with an Accucore PFP column (150 \times 2.1 mm, 2.6- μm particle size; Thermo-Scientific) at a flow rate of 0.2 mL/min. Elution started with 99% of mobile phase A (0.1% formic acid in water) and 1% mobile phase B (100% acetonitrile) for 2 minutes, followed by an 18-minute linear gradient to 50% of phase A, a 1-minute linear gradient to 30% of phase A, and a 1-minute linear gradient to 99% of phase A, which was maintained for 12 minutes to equilibrate the column. The total run time was 35 minutes. The mass spectrometric analysis was performed using a Micromass Quattro Micro API triple-quadrupole (Waters Corp) in positive ion mode and multiple-reaction monitoring mode, measuring the fragmentation products of the deprotonated pseudomolecular ions. The choice of fragmentation products for all compounds and the optimization of collision-induced dissociation energies were done in continuous-flow mode using standard solutions at concentrations of 10 ng/ μL for all compounds. Data were processed with the MassLynx software (Waters Corp). Plasma concentrations of TRP and KP metabolites were quantified by reference to 5-point calibration curves that were always run in parallel and that were linear over the used concentration ranges (15 to 118 $\mu\text{mol/L}$ for TRP, 0.4 to 3.8 $\mu\text{mol/L}$ for KYN, 16 to 127 nmol/L for KYNA, and 20 to 157 nmol/L for 3-HAA). The r^2 values were always >0.95 . The limit of detection (ie, 3 times the background) was 1 nmol/L for TRP, KYN, and KYNA and 10 nmol/L for 3-HAA. The quality of analytical results was checked by assaying quality-control samples, which were always within 20% error. Plasma concentrations of TRP and KYN were expressed in micromolar, and KYNA and 3-HAA were expressed in nanomolar.

Plasma levels of C-reactive protein were measured using the latex immunoassay CRP Vario -high-sensitivity method with the Ci16200 Architect instrument (Abbott Diagnostics, USA).

Data Collection

The participating hospitals were part of the Finnish Intensive Care Consortium, and 20 of 21 ICUs used the same electronic data management system and data validation software (Web Validator; Tieto Corp). Data from study patients were

collected prospectively using an Internet-based case report form. Prehospital data were collected by the paramedics in accordance with the Utstein Style guidelines and included whether or not the arrest was witnessed, the administration of bystander-initiated life support, the time from call to the dispatch center to return of spontaneous circulation (ROSC), and the use of adrenaline.¹⁵ In-hospital care data were collected electronically and comprised the Simplified Acute Physiology Score II score and ICU and hospital mortality. For the present trial, we used Simplified Acute Physiology Score II score–derived systolic blood pressure and bicarbonate to define the severity of post-CA shock.

Survival and 12-Month Neurological Outcome

Time of death was recorded for each patient. A specialist in neurology (M.T.) blinded to management in the ICU contacted patients discharged from the hospital by phone 1 year after CA and determined neurological outcome according to the Pittsburgh Cerebral Performance Categories¹⁴; we defined 12-month good outcome as category 1 or 2 and 12-month poor outcome as categories 3–5.

Statistical Analysis

Baseline characteristics by outcome occurrence were compared by chi-square test in case of categorical variables or by *t* test or nonparametric Wilcoxon test for continuous variables. Comparison in baseline characteristics and KP variables between healthy volunteers and study patients were performed by Fisher chi-square test or by Wilcoxon test. When the subgroups of ICU death and ICU survival were compared with healthy volunteers, adjustment for the 2 contrasts was applied. Median values with interquartile ranges (IQRs) were calculated for KP metabolites. Levels of KP metabolites across variables at admission were compared by the Wilcoxon test and the Kruskal-Wallis test, and changes over time were analyzed using the Wilcoxon signed rank test for paired data. Multivariable linear regression was used to identify the independent factors at resuscitation influencing metabolite levels at ICU admission. Results of linear regression are reported in terms of β coefficients with 95% CIs and *P* values. Multivariable logistic regression was used to identify factors at resuscitation that were predictors of ICU mortality and poor outcome at 12 months from the arrest. All variables associated with the outcome in the univariate analysis (*P*<0.05) were included in the multivariable model. Each KP metabolite was included separately in the model as a continuous variable. Odds ratios (ORs) with the corresponding 95% CIs were calculated, and *P* values were considered statistically significant if they were <0.05. The addition of new predictive variables (ie, KP metabolites) into a model containing clinical

risk factors (basic model) was assessed by comparing the area under the curve by DeLong comparison.¹⁶ The increase in the discriminative value of each KP metabolite for ICU mortality and long-term outcome was assessed by the category-free Net Reclassification Index.¹⁷ All statistical analyses were performed with SAS software, version 9.2 (SAS Institute).

Results

The FINNRESUSCI study included 548 patients.¹⁴ Among these, informed consent for blood sampling at ICU admission was obtained for 245 patients, and all were included in the study. Differences between these 245 patients and the 303 not included because of the absence of the blood sample and/or consent are reported in Table 1. Of the 245 patients included in the study, 198 (81%) survived to ICU discharge, 144 (59%) survived to hospital discharge, and 124 (51%) were alive at 12 months. Of the 124 patients who survived to 12 months, 116 (94% of survivors and 47% of the total 245 resuscitated patients) had a favorable neurological outcome (Pittsburgh Cerebral Performance Category 1 or 2) at 12 months.

Among the 245 patients, 156 (64%) had a CA with an initial shockable rhythm, whereas 88 (36%) were nonshockable (data on initial rhythm was missed for 1 patient). Among the shockable CA patients, 140 (90%) survived to ICU discharge and 93 (60%) had a good 12-month neurological outcome. Among the nonshockable CA patients, 58 (66%) survived to ICU discharge and 23 (26%) had a good 12-month neurological outcome. Baseline characteristics and factors influencing ICU survival and 12-month outcome are shown in Table 2. Prehospital factors univariately associated with higher likelihood of ICU survival and 12-month outcome were younger age, a shockable initial rhythm, witnessed CA, no use of adrenaline, and a shorter time to ROSC (Table 2).

KP Metabolites and Factors Related to Resuscitation

The median levels of TRP and KP metabolites in the 10 healthy volunteers were comparable to those in the literature (TRP: 82.8 μ mol/L [IQR: 70.0 to 89.0]; KYN: 2.4 μ mol/L [IQR: 2.3 to 2.9]; KYN/TRP: 0.03 [IQR: 0.027 to 0.034]; KYNA: 40.6 nmol/L [IQR: 32.5 to 60.3]; 3-HAA: 24.3 nmol/L [IQR: 18.1 to 27.8]).^{6,18} There were no differences in the basic characteristics between the healthy volunteers and the resuscitated patients (Table 3). TRP was significantly lower and the ratio of KYN to TRP was significantly higher in all resuscitated patients compared with healthy volunteers. When patients who did not survive to ICU discharge were

Table 1. Baseline Characteristics and Clinical Factors at Resuscitation Between Patients With a Blood Sample and Included in the Analysis and Those Without a Blood Sample and Not Included in the Analysis

Characteristic	Patients With Blood Sample (n=245)	Patients Without Blood Sample (n=303)	P Value
Age, y, median (IQR)	63 (53 to 72)	63 (56 to 72)	0.566
Sex, male, n (%)	197 (80)	217 (72)	0.013
Shockable rhythm, n (%)	156 (64)	152 (50)	0.001
Witnessed cardiac arrest, n (%)	222 (91)	267 (88)	0.437
BLS, n (%)	140 (57)	152 (50)	0.112
Adrenaline used, n (%)	163 (67)	201 (69)	0.822
Time to ROSC, min, median (IQR)	20 (13 to 28)	20 (13 to 27)	0.935
Therapeutic hypothermia, n (%)	176 (72)	135 (45)	0.001
SAPS II score, median (IQR)	60 (47 to 69)	59 (44 to 68)	0.691
ICU survival, n (%)	198 (81)	231 (76)	0.196
12-month survival, n (%)	124 (51)	123 (40)	0.001
Good outcome at 12 months, n (%)	116 (47)	97 (32)	0.001

BLS indicates bystander-initiated basic life support; ICU, intensive care units; IQR, interquartile range; ROSC, return of spontaneous circulation; SAPS II, Simplified Acute Physiology Score II.

considered, all KP metabolites and the ratio of KYN to TRP were significantly higher compared with healthy volunteers (Table 3).

At ICU admission, median KP metabolite values were as follows: TRP: 39.5 $\mu\text{mol/L}$ (IQR: 30.8 to 50.4); KYN: 2.6 $\mu\text{mol/L}$ (IQR: 1.8 to 3.5); KYN/TRP: 0.064 (IQR: 0.045 to 0.089); KYNA: 52.8 nmol/L (IQR: 27.5 to 91.8); 3-HAA: 28.1 nmol/L (IQR: 17.5 to 51.2). At ICU admission, the levels of KYN, KYN to TRP, and KYNA were higher in older patients (Table 4). All KP metabolites were also significantly higher, except for TRP and KYNA, which were significantly lower, in patients with an initial nonshockable rhythm compared with those with a shockable rhythm (Table 4). Longer time to ROSC accounted for significantly higher levels of KYNA and

3-HAA (Table 4). By linear regression models, presence of a shockable rhythm and use of adrenaline were the main independent predictors of levels of KP metabolites, as detailed in Table 5.

On average, plasma C-reactive protein levels were 2.8 mg/L (IQR: 1.2 to 8.9), with no difference between patients who survived to ICU (2.8 mg/L [IQR: 1.2 to 8.3]) and those who died (2.8 mg/L [IQR: 1.2 to 23], $P=0.488$).

Correlations Between KP Metabolites and Severity of Shock

Levels of all KP metabolites and KYN to TRP at ICU admission were significantly higher in patients who had lower systolic

Table 2. Baseline Characteristics and Clinical Factors at Resuscitation Between ICU Survivors and Nonsurvivors and Patients With Good and Poor Outcome at 12 Months

Characteristic	ICU Survival (n=198)	ICU Death (n=47)	Good Outcome at 12 Months (n=116)	Poor Outcome at 12 Months (n=128)
Age, median (IQR)	63 (56 to 72)	65 (59 to 75)	61 (53 to 67) [§]	66 (59 to 75)
Sex, male, n (%)	159 (80)	38 (81)	92 (79)	105 (82)
Shockable rhythm, n (%)	140 (71)**	16 (34)	93 (80) [§]	63 (50)
Witnessed cardiac arrest, n (%)	182 (92)	40 (85)	111 (96) [§]	110 (86)
BLS, n (%)	117 (59)	23 (49)	73 (63) [†]	67 (52)
Adrenaline used, n (%)	120 (61)**	43 (91)	55 (48) [§]	107 (84)
Time to ROSC, min, median (IQR)	19 (12 to 26)**	24 (20 to 31)	16 (11 to 22) [§]	24 (18 to 31)
Therapeutic hypothermia, n (%)	149 (75)*	27 (57)	89 (77)	87 (68)

[†]Data on 1-year outcome was missed for 1 patient. * $P<0.05$ and ** $P<0.01$ vs ICU death; [§] $P<0.01$ vs poor outcome at 12 months. BLS indicates bystander-initiated basic life support; ICU, intensive care units; IQR, interquartile range.

Table 3. Comparison Between Baseline Characteristics and Kynurenine Pathway Metabolites in the Healthy Volunteers and in Patients Resuscitated From Cardiac Arrest

	Healthy Volunteers (n=10)	Cardiac Arrest Patients (n=245)	P Value vs Healthy Volunteers	ICU Survival (n=198)	P Value vs Healthy Volunteers*	ICU Death (n=47)	P Value vs Healthy Volunteers*
Age, y	63 (59 to 65)	63 (56 to 72)	0.86	63 (56 to 72)	0.98	65 (59 to 75)	0.437
Sex, male, n (%)	8 (80)	197 (80.4)	1.00	159 (80.3)	1.00	38 (80.6)	1.000
TRP, $\mu\text{mol/L}$	82.8 (70.0 to 89.0)	39.5 (30.8 to 50.4)	<0.0001	40.1 (31.8 to 51.2)	<0.0001	37.1 (25.0 to 44.8)	<0.0001
KYN, $\mu\text{mol/L}$	2.4 (2.3 to 2.9)	2.6 (1.8 to 3.5)	0.624	2.4 (1.7 to 3.1)	0.874	3.8 (2.5 to 4.5)	0.003
Ratio of KYN to TRP	0.03 (0.027 to 0.034)	0.064 (0.045 to 0.089)	<0.0001	0.059 (0.043 to 0.079)	<0.0001	0.095 (0.067 to 0.122)	<0.0001
KYNA, nmol/L	40.6 (32.5 to 60.3)	52.8 (27.5 to 91.8)	0.542	49.2 (26.7 to 83.6)	0.794	90.6 (43.3 to 191.8)	0.058
3-HAA, nmol/L	24.3 (18.1 to 27.8)	28.1 (17.5 to 51.2)	0.277	24.7 (16.7 to 42.4)	0.519	54.6 (28.5 to 104.5)	0.007

Data are reported as median (interquartile range) except as indicated. 3-HAA indicates 3-hydroxyanthranilic acid; ICU, intensive care units; KYN, kynurenine; KYNA, kynurenic acid; TRP, tryptophan.

*P considered significant if <0.025.

blood pressure and lower levels of bicarbonate during the subsequent 24 hours after ROSC (Table 4).

KP Metabolites at ICU Admission and Outcome

All KP metabolites except TRP correlated with ICU death (Figure 1) and with 12-month death and neurological outcome (Figure 2 and Table 4). Levels of all KP metabolites and the ratio of KYN to TRP were significantly higher in patients who died compared with those who survived (Figures 1 and 2) and in patients with poor outcomes at 12 months compared with those with good outcomes (Table 4). The ORs for prediction of ICU mortality were 0.982 (95% CI: 0.959 to 1.004 per $\mu\text{mol/L}$ increase; $P=0.11$) for TRP, 1.589 (95% CI: 1.287 to 1.963 per $\mu\text{mol/L}$ increase; $P<0.0001$) for KYN, 2.284 (95% CI: 1.518 to 3.438 per 1 SD increase; $P<0.0001$) for ratio of KYN to TRP, 1.004 (95% CI: 1.002 to 1.007 per nmol/L increase; $P=0.0003$) for KYNA, and 1.019 (95% CI: 1.010 to 1.027 per nmol/L increase; $P<0.0001$) for 3-HAA. The ORs for the prediction of 12-month poor outcome were 0.987 (95% CI: 0.970 to 1.004 per $\mu\text{mol/L}$ increase; $P=0.15$) for TRP, 1.529 (95% CI: 1.233 to 1.896 per $\mu\text{mol/L}$ increase; $P=0.0001$) for KYN, 2.569 (95% CI: 1.560 to 4.231 per 1 SD increase; $P=0.0002$) for ratio of KYN to TRP, 1.004 (95% CI: 1.001 to 1.007 per nmol/L increase; $P=0.006$) for KYNA, and 1.017 (95% CI: 1.008 to 1.027 per nmol/L increase; $P=0.0002$) for 3-HAA.

We developed multivariable models for the prediction of ICU mortality and 12-month poor outcome. The clinical (basic) model for prediction of ICU death included the initial CA rhythm (shockable or nonshockable), time to ROSC, use of adrenaline, and whether or not therapeutic hypothermia was applied (Table 2). Each of the KP metabolites and the ratio of

KYN to TRP, but not TRP, were independently associated with ICU death when added to the basic model (Table 6). The addition of each KP metabolite to the basic model improved the prognostic discrimination for ICU death, significantly increasing the area under the receiver operating characteristic curve, and reclassification, estimated by category-free net reclassification index. Comparison between the areas under the curve of the basic model and those of the incremental models are reported in Table 7, together with the category-free net reclassification index for each KP metabolite.

The basic model for prediction of poor outcome at 12 months included age, whether or not the CA was witnessed, the initial CA rhythm (shockable or nonshockable), time to ROSC, and use of adrenaline (Table 2). When added to the basic model, only KYNA and 3-HAA were associated with poor outcome at 12 months (Table 6). The incremental models with the addition of KYNA or 3-HAA significantly improved reclassification for prediction of 12-month poor outcome (Table 7).

Discussion

The present study showed that the KP is activated early after resuscitation from out-of-hospital CA. Patients who died before ICU discharge exhibited significantly higher levels of KP metabolites at ICU admission compared with those who survived. All KP metabolite levels predicted ICU death after resuscitation, whereas KYNA and 3-HAA were associated with 12-month poor neurological outcome. KP activation was higher in patients resuscitated from a nonshockable CA compared with those resuscitated from a shockable CA.

Table 4. Kynurenine Pathway Activation by Age, Cardiac Arrest Presenting Rhythm, Duration of Cardiac Arrest, and Patient Characteristics

Variable	TRP, $\mu\text{mol/L}$	KYN, $\mu\text{mol/L}$	Ratio of KYN of TRP	KYNA, nmol/L	3-HAA, nmol/L
<i>Admission levels</i>					
Age, y					
<59	38.4 (31.7 to 49.8)	2.2 (1.6 to 3.0)	0.05 (0.04 to 0.08)	42.3 (21.2 to 80.5)	23.9 (17.8 to 49.2)
59 to 68	39.8 (30.9 to 47.9)	2.4 (1.7 to 3.2)	0.06 (0.04 to 0.08)	50.5 (26.7 to 90.6)	29.2 (17.8 to 58.5)
>68	41.1 (28.8 to 51.6)	3.1 (2.4 to 4.1)	0.07 (0.06 to 0.1)	64.6 (37.9 to 100.1)	29.7 (16.9 to 46.1)
<i>P</i> value	0.849	<0.0001	0.0006	0.0298	0.5488
<i>Cardiac arrest presenting rhythm</i>					
Shockable	42.4 (34.1 to 53.1)	2.4 (1.7 to 3.2)	0.06 (0.04 to 0.08)	57.3 (38.9 to 100.3)	24.7 (16.9 to 42.0)
Nonshockable	33.1 (24.8 to 42.3)	2.9 (1.7 to 3.2)	0.08 (0.06 to 0.12)	31.6 (15.3 to 82.8)	33.7 (21.4 to 72.4)
<i>P</i> value	0.0001	0.0282	0.0001	0.0001	0.0056
<i>Time to ROSC, min</i>					
1 to 15	39.8 (30.4 to 51.3)	2.4 (1.6 to 3.2)	0.06 (0.04 to 0.09)	40.8 (23.9 to 77.6)	22.2 (13.3 to 35.8)
16 to 24	40.2 (29.0 to 50.0)	2.6 (1.8 to 3.9)	0.06 (0.04 to 0.1)	54.6 (29.4 to 93.0)	31.7 (19.8 to 58.6)
25 to 57	38.2 (32.0 to 49.8)	2.6 (2.0 to 3.4)	0.07 (0.05 to 0.08)	57.3 (34.9 to 101.2)	29.8 (18.6 to 59.8)
<i>P</i> value	0.901	0.206	0.185	0.0294	0.0046
<i>Lowest 24-hour systolic blood pressure, mm Hg</i>					
≥ 100	42.4 (33.3 to 57.1)	2.3 (1.7 to 3.1)	0.05 (0.04 to 0.07)	34.9 (20.8 to 83.9)	24.5 (15.0 to 49.3)
70 to 99	39.0 (30.8 to 49.6)	2.5 (1.8 to 3.2)	0.06 (0.04 to 0.09)	52.9 (29.3 to 87.0)	25.8 (17.6 to 44.6)
<70	36.6 (28.3 to 48.4)	3.7 (2.9 to 4.7)	0.1 (0.07 to 0.14)	90.6 (27.2 to 384.8)	66.6 (19.5 to 109.2)
<i>P</i> value	0.156	0.0002	<0.0001	0.0141	0.009
<i>24-hour HCO₃, mEq/L</i>					
≥ 20	39.8 (33.1 to 51.6)	2.2 (1.7 to 3.0)	0.05 (0.04 to 0.08)	45.7 (26.6 to 68.9)	24.7 (16.1 to 44.6)
15 to 19	40.1 (29.1 to 50.0)	2.6 (2.0 to 3.7)	0.07 (0.05 to 0.09)	52.8 (26.6 to 99.7)	28.3 (18.6 to 44.4)
<15	33.3 (28.3 to 44.8)	3.2 (2.9 to 4.3)	0.1 (0.07 to 0.16)	95.6 (66.5 to 349.4)	72.4 (28.9 to 119.8)
<i>P</i> value	0.273	<0.0001	<0.0001	<0.0001	0.0001
<i>Outcome at 12 months</i>					
Good	39.9 (33.2 to 52.6)	2.2 (1.7 to 3.0)	0.05 (0.04 to 0.08)	49.2 (26.7 to 80.0)	23.6 (16.5 to 38.3)
Poor	39.0 (29.0 to 48.2)	2.9 (2.2 to 4.1)	0.07 (0.06 to 0.1)	58.7 (28.4 to 100.6)	33.9 (17.9 to 66.5)
<i>P</i> value	0.1224	<0.0001	<0.0001	0.046	0.002

Data are reported as median (interquartile range). 3-HAA indicates 3-hydroxyanthranilic acid; KYN, kynurenine; KYNA, kynurenic acid; ROSC, return of spontaneous circulation; TRP, tryptophan.

In our population, TRP was significantly lower and ratio of KYN to TRP was significantly higher in all resuscitated patients compared with the 10 healthy volunteers. Of interest is the observation that levels of KYN, KYNA, and 3-HAA in our healthy volunteers were not significantly different from those of ICU survivors, although they were significantly lower compared with patients who failed to survive to ICU discharge. These results are further evidence that early KP activation, expressed by the ratio of KYN to TRP, is always present after resuscitation from CA compared with healthy volunteers. Nevertheless, KP metabolites KYN, KYNA, and 3-

HAA were markedly increased in the instance of poor outcome, supporting the specific prognostic role of KP.

KP is a major pathway of the catabolism of the essential amino acid TRP and is activated during inflammation and tissue injury. On inflammatory stimulation, the enzyme indoleamine 2,3-dioxygenase is induced, and the consequent KP activation results in degradation of TRP into KYN.^{9,19} A systemic inflammatory and immune response is observed after CA, and the magnitude of the inflammatory response correlates with the severity of post-CA circulatory shock and, ultimately, with outcome.⁴ The inflammatory response is

Table 5. Independent Predictors of Levels of Kynurenine Pathway Metabolites

Metabolite	Variable	β	P Value
TRP	Age	-0.539	0.640
	Sex (male)	2.731	0.247
	Shockable rhythm	8.684	<0.0001
	BLS	-0.007	0.997
	Adrenaline	-2.976	0.208
	Time to ROSC	0.659	0.629
KYN	Age	0.417	0.002
	Sex (male)	0.558	0.039
	Shockable rhythm	-0.263	0.268
	BLS	-0.428	0.057
	Adrenaline	0.653	0.016
	Time to ROSC	0.015	0.923
Ratio of KYN to TRP	Age	0.264	0.0004
	Sex (male)	0.179	0.233
	Shockable rhythm	-0.506	0.0002
	BLS	-0.244	0.052
	Adrenaline	0.395	0.0092
	Time to ROSC	-0.014	0.875
KYNA	Age	5.276	0.589
	Sex (male)	-9.476	0.634
	Shockable rhythm	40.29	0.022
	BLS	-24.76	0.136
	Adrenaline	50.53	0.012
	Time to ROSC	2.037	0.859
3-HAA	Age	-0.024	0.994
	Sex (male)	-3.338	0.621
	Shockable rhythm	-19.76	0.001
	BLS	-3.005	0.592
	Adrenaline	5.981	0.376
	Time to ROSC	5.566	0.154

Multivariable linear regression model. 3-HAA indicates 3-hydroxyanthranilic acid; BLS, bystander-initiated life support; KYN, kynurenine; KYNA, kynurenic acid; ROSC, return of spontaneous circulation; TRP, tryptophan.

commonly observed after resuscitation from CA and seems to be related to the duration of ischemia.⁴ Adrie and colleagues⁴ showed that the magnitude of such post-CA inflammatory response is similar to that observed in severe sepsis and correlates with lactate levels. In their study, however, the levels of various pro-inflammatory cytokines were not independent predictors of survival. The ratio of KYN to TRP is regarded as an indicator of indoleamine 2,3-dioxygenase activity and has been shown to predict death and severity of shock in bacteremic patients.^{18,19} Huttunen et al reported

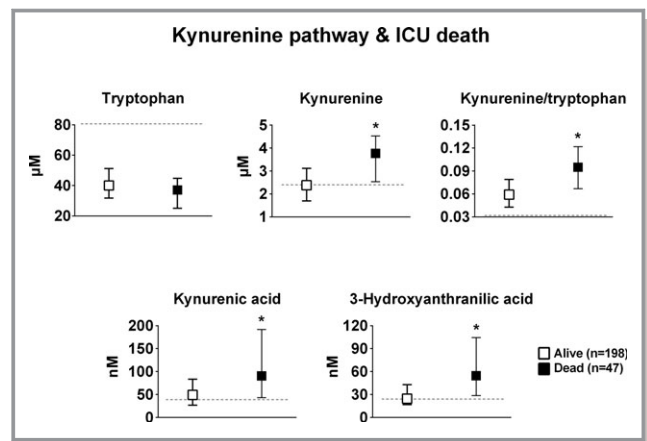


Figure 1. Tryptophan and kynurenine pathway metabolites and intensive care unit death. Data are reported as median and interquartile range. Gray dashed lines represent the median values of each metabolite in healthy volunteers. * $P < 0.001$ vs survivors. ICU indicates intensive care unit.

similar levels of TRP but higher levels of KYN and KYN to TRP in patients with fatal bacteremia.²⁰

Earlier studies have also reported that elevated concentrations of C-reactive protein or other inflammatory mediators (eg, procalcitonin) correlated with patients' clinical states and with prediction of survival.^{21,22} Circulating concentrations of these biomarkers, however, rise slowly, achieving values predictive of outcome after 12 to 24 hours following resuscitation. In the current study, patients exhibiting high KP activation at ICU admission subsequently had more severe shock and were more likely to die in the ICU. Accordingly, all KP metabolites were independent predictors of early death. More important, KYNA and 3-HAA seem to have the potential to further predict long-term neurological outcome after CA. At

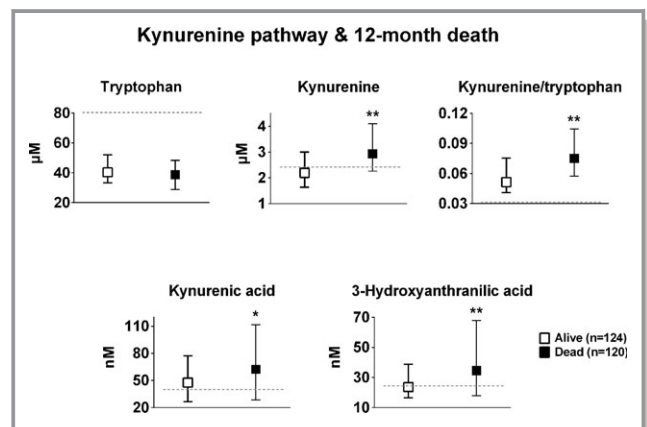


Figure 2. Tryptophan and kynurenine pathway and 12-month death. Data are reported as median and interquartile range. Gray dotted lines represent the median values of each metabolite in healthy volunteers. * $P < 0.02$ and ** $P \leq 0.001$ vs survivors.

Table 6. Multivariable Logistic Models for the Prediction of ICU Mortality and 12-Month Poor Outcome

	OR	95% CI	P Value
ICU death			
TRP	0.994 per 1 $\mu\text{mol/L}$ increase	0.970 to 1.018	0.614
KYN	1.431 per 1 $\mu\text{mol/L}$ increase	1.136 to 1.803	0.002
KYN to TRP	1.665 per 1 SD increase	1.143 to 2.426	0.0079
KYNA	1.005 per 1 nmol/L increase	1.002 to 1.007	0.0007
3-HAA	1.013 per 1 nmol/L increase	1.005 to 1.021	0.0015
12-month poor outcome			
TRP	1.007 per 1 $\mu\text{mol/L}$ increase	0.985 to 1.030	0.519
KYN	1.249 per 1 $\mu\text{mol/L}$ increase	0.982 to 1.588	0.069
KYN to TRP	1.251 per 1 SD increase	0.798 to 1.963	0.329
KYNA	1.004 per 1 nmol/L increase	1.001 to 1.008	0.0139
3-HAA	1.011 per 1 nmol/L increase	1.001 to 1.022	0.0272

3-HAA indicates 3-hydroxyanthranilic acid; ICU, intensive care unit; KYN, kynurenine; KYNA, kynurenic acid; OR, odds ratio; TRP, tryptophan.

ICU admission, plasma levels of C-reactive protein were still low and thus were not predictive of outcome.

The KP can be activated in the periphery as well as in the nervous system. Peripheral KYN readily crosses the blood–brain barrier and is taken up by glial cells.²³ In the central nervous system further metabolism is segregated and under physiologic conditions; after the influx of KYN into the brain, equal amounts of KYNA and 3-HAA are produced.²⁴ These

downstream metabolites, KYNA and 3-HAA, have been proposed as important modulators of neurologic injury.⁶ Indeed, 3-HAA exerts neurotoxic actions by inducing both cerebral oxidative stress and excitotoxicity through activation of N-methyl-D-aspartate receptors, whereas KYNA has neuroprotective properties related to its activity as an N-methyl-D-aspartate antagonist.²⁵ In patients with stroke, KP activation has been shown to correlate with stroke severity

Table 7. Comparison Between the Basic Model and the Incremental Models With the Addition of Kynurenine Pathway Metabolites for Prediction of ICU Death and 12-Month Poor Outcome

	ROC-AUC	Δ ROC-AUC (SE)	P Value	cfNRI (SE)	P Value
ICU death					
Basic model	0.78				
TRP	0.78	−0.001 (0.004)	0.788	0.20 (0.16)	0.21
KYN	0.83	0.046 (0.017)	0.009	0.50 (0.16)	0.002
KYN to TRP	0.81	0.023 (0.012)	0.016	0.46 (0.16)	0.005
KYNA	0.83	0.051 (0.021)	0.015	0.54 (0.16)	0.001
3-HAA	0.82	0.042 (0.020)	0.037	0.65 (0.16)	<0.0001
12-month poor outcome					
Basic model	0.83				
TRP	0.84	0.003 (0.002)	0.256	−0.04 (0.13)	0.738
KYN	0.84	0.005 (0.006)	0.383	0.11 (0.13)	0.392
KYN to TRP	0.83	0.000 (0.003)	0.975	−0.07 (0.13)	0.588
KYNA	0.84	0.012 (0.008)	0.114	0.26 (0.12)	0.046
3-HAA	0.84	0.009 (0.007)	0.212	0.31 (0.12)	0.017

Δ indicates the difference in ROC-AUC between the basic model and the model with the addition of kynurenine pathway metabolites; 3-HAA indicates 3-hydroxyanthranilic acid; cfNRI, category-free net reclassification index; KYN, kynurenine; KYNA, kynurenic acid; ROC-AUC, area under the receiver operating characteristic curve; TRP, tryptophan.

and cerebral infarct size.⁶ Brouns showed that the ratio of KYN to TRP on hospital admission correlated with poor functional outcome at 3 months.²⁶ In the present study, the ratio of KYN to TRP was higher in patients with a poor outcome at 12 months, and the absolute levels were comparable to those reported by Brouns and Darlington (ie, between 0.04 and 0.09).^{6,26}

Interestingly, in our study, both KYNA and 3-HAA increased similarly, and their increases were more significant in the instances of longer duration of CA and, thereby, greater ischemic insult. Although KYNA generation might represent a protective adaptive response to overcome the neurotoxic effects resulting from 3-HAA,²⁵ the ratio of KYNA to 3-HAA was not significantly different in patients who survived compared with those who died and did not correlate with outcomes (data not shown). Accordingly, relationships between KYNA and 3-HAA and between KYNA and neuroprotection still are not fully understood. Patients resuscitated from nonshockable CA showed greater activation of the KP, having significantly higher levels of all KP metabolites but lower neuroprotective KYNA compared with patients resuscitated from shockable CA.

In sepsis, high KP activity is associated with the severity of shock and with a vasopressor requirement.⁵ Level of KP activation, expressed as the ratio of KYN to TRP, in our study was comparable to KP activation in septic patients with a fairly low requirement for inotropes. Inotrope requirement during post-CA syndrome care is generally fairly low, and high requirement correlates with poor outcome.²⁷ In contrast, in stroke patients, it is intriguing that hypotension is uncommon despite high activation of the KP.²⁸ This finding contrasts with the hypothesis that activation of the KP per se causes vasodilatory shock and hypotension. We cannot define whether KP activation after CA is an epiphenomenon or a causative pathway involved in postresuscitation severe hypotension and, ultimately, in outcome. Nevertheless, additional evidence of the involvement of the KP in cardiovascular pathology has been reported recently in 3224 patients suffering from stable coronary artery disease.⁸ In these patients, KP activation was a strong predictor of occurrence of major coronary events, acute myocardial infarction, and cardiovascular mortality.⁸ The interplay among shock, neurological damage, and systemic inflammation seems to be complex, and further studies should examine where organ-specific activation of the KP occurs.

We limited our study to patients with blood samples, who represented approximately half of the whole data set of the FINNRESUSCI study.¹⁴ Some differences existed between the 245 patients included in the study and the 303 not included, and patients with blood samples were somewhat less severely ill than those without blood samples. Nevertheless, early KP activation was consistently observed after resuscitation in

patients who presented poor early and long-term outcome. The strengths of this study include the sample size, which makes it one of the largest biohumoral studies in out-of-hospital CA; the prospective acquisition of clinical data; and the determination of outcome performed prospectively by a qualified independent assessor.

In conclusion, higher levels of KP metabolites at ICU admission are associated with hypotension during the first 24 hours after resuscitation and with ICU death in out-of-hospital CA patients. In addition, KYNA and 3-HAA independently predict poor 12-month neurological outcome. Further studies may elucidate whether the KP contributes to post-CA syndrome and whether it may be amenable to targeted therapeutic interventions.

Appendix

Finnresusci Investigators

The FINNRESUSCI Laboratory Study Group: Participating hospitals, investigators (Inv.) and study nurses (SN.) in the FINNRESUSCI study.

Satakunta Central Hospital, Dr Vesa Lund (Inv.), Päivi Tuominen, Satu Johansson, Pauliina Perkola, Elina Kumpulainen (SN.); East Savo Central Hospital, Dr Markku Suvela (Inv.), Sari Hirvonen, Sirpa Kauppinen (SN.); Central Finland Central Hospital, Dr Raili Laru-Sompa (Inv.), Mikko Reilama (SN.); South Savo Central Hospital, Dr Heikki Laine (Inv.), Pekka Kettunen, Iina Smolander (SN.); North Carelia Central Hospital, Dr Matti Reinikainen (Inv.), Tero Surakka (SN.); Seinäjoki Central Hospital, Dr Kari Saarinen (Inv.), Pauliina Lähdeaho, Johanna Soini (SN.); South Carelia Central Hospital, Dr Seppo Hovilehto (Inv.); Vaasa Central Hospital, Dr Simo-Pekka Koivisto, Dr Raku Hautamäki (Inv.); Kanta-Häme Central Hospital, Dr Ari Alaspää (Inv.), Tarja Heikkilä (SN.); Lappi Central Hospital, Dr Outi Kiviniemi (Inv.), Esa Lintula (SN.); Keski-Pohjanmaa Central Hospital, Dr Tadeusz Kaminski (Inv.), Jane Roiko (SN.); Kymenlaakso Central Hospital, Dr Seija Alila, Dr Jussi Pentti (Inv.), Reija Koskinen (SN.); Länsi-Pohja's Central Hospital, Dr Jorma Heikkinen (Inv.) Helsinki University Hospital, Jorvi Hospital, Dr Jukka Vaahersalo, Dr Tuomas Oksanen, Dr Tero Varpula (Inv.), Anne Eronen, Teemu Hult, Taina Nieminen (SN.); Meilahti Hospital Medical ICU, Dr Tom Bäcklund (Inv.), Leevi Kauhanen (SN.); Meilahti Hospital ICU, Dr Kirsi-Maija Kaukonen, Dr Ville Pettilä (Inv.), Leena Pettilä, Sari Sutinen (SN.); Tampere University Hospital, Dr Sanna Hoppu, Dr Jyrki Tenhunen, Dr Sari Karlsson (Inv.), Atte Kukkurainen, Simo Varila, Samuli Kortelainen, Minna-Liisa Peltola (SN.); Kuopio University Hospital, Dr Pamela Hiltunen, Dr Jouni Kurola, Dr Esko Ruokonen (Inv.), Elina Halonen, Saija Rissanen, Sari Rahikainen (SN.); Oulu

University Hospital, Dr Risto Ahola, Dr Tero Ala-Kokko (Inv.), Sinikka Sälkiö (SN.)

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Disclosures

None.

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