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Secretoneurin as a marker for hypoxic brain injury after cardiopulmonary resuscitation

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Take home message: The neuropeptide secretoneurin is a biomarker for hypoxic brain injury which is rapidly released in the first 24 h after cardiopulmonary resuscitation. Owing to its specific kinetics and properties, secretoneurin may significantly improve prediction of neurological outcome in combination with the existing marker neuron-specific enolase. Further studies in the context of SN and CPR are warranted to confirm these results.

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Abstract *Purpose:* The neuropeptide secretoneurin (SN) shows widespread distribution in the brain. We evaluated whether SN is elevated after cardiopulmonary resuscitation (CPR) and could serve as a potential new biomarker for hypoxic brain injury after CPR. *Methods:* This was a prospective observational clinical study. All patients admitted to a tertiary medical intensive care unit after successful CPR with expected survival of at least 24 h were consecutively enrolled from September 2008 to April 2013. Serum SN and neuron-specific enolase were determined in 24 h intervals starting with the day of CPR for 7 days. Neurological outcome was assessed with the Cerebral Performance Categories Scale (CPC) at hospital discharge. *Results:* A total of 134 patients were included with 49 % surviving to good neurological outcome (CPC 1–2). SN serum levels peaked within the first 24 h showing on average a sixfold increase above normal. SN levels were significantly higher in patients with poor (CPC 3–5) than in patients with good neurological outcome [0–24 h: 75 (43–111) vs. 38 (23–68) fmol/ml, $p < 0.001$; 24–48 h: 45 (24–77) vs. 23 (16–39) fmol/ml, $p < 0.001$]. SN determined within the first 48 h showed a receiver operating characteristic (ROC) area under the curve (AUC) of 0.753 (0.665–0.841). NSE in the first 72 h had a ROC-AUC of 0.881 (0.815–0.946). When combining the two biomarkers an AUC of 0.925 (0.878–0.972) for outcome prediction could be reached. *Conclusions:* SN is a promising early biomarker for hypoxic brain injury. Further studies will be required for confirmation of these results.

Keywords Cardiopulmonary resuscitation · Hypothermia · Hypoxia · Secretoneurin · Neuron-specific enolase · Cerebral Performance Categories Scale

Introduction

Major advances in resuscitation practice and post-resuscitation care have improved survival and neurological outcome after cardiac arrest (CA) over the last decade [1, 2]. Survival is usually reported in a range from 7 to 22 % in out-of-hospital and in-hospital CA [1, 3, 4] with better rates shown for patients with a shockable rhythm and treated with controlled hypothermia [5]. Significant improvements and standardization in post-resuscitation care over the last decade could enable that about half of the patients with return of spontaneous circulation (ROSC) can expect to survive hospitalization [6, 7].

This implies that the early and precise evaluation of neurological damage is of great importance [8] to determine further therapeutic strategies with serum biomarkers playing an important role. Several markers of hypoxic brain injury have been investigated of which neuron-specific enolase (NSE) has been become widely established [9, 10]. However, results still remain conflicting concerning the best time point for determination, optimal cut-off levels and their predictive properties when mild therapeutic hypothermia (MTH) is applied [9, 11, 12].

Secretoneurin (SN) represents a 33 amino acid neuropeptide that is specifically expressed in endocrine, neuroendocrine and neuronal tissues. SN shows a widespread distribution in the brain. Although present in very low concentrations in peripheral blood of healthy individuals (less than 9 fmol/ml), SN was detected in the cerebrospinal fluid at a much higher concentration than determined for other "classical" neuropeptides [13, 14]. SN exerts a variety of biological functions like induction of angiogenesis and growth of new blood vessels and is pronouncedly upregulated by hypoxia. Under these conditions expression of SN can also be induced in non-endocrine tissues like muscle cells, pneumocytes or tumour epithelial cells [15].

The aim of our study was to evaluate the role of SN as a new potential marker for severity of hypoxic brain injury in patients admitted to the intensive care unit (ICU) after successful cardiopulmonary resuscitation (CPR).

Methods

We conducted a prospective observational trial including all consecutive adult patients (age at least 18 years) admitted to the medical ICU of the University Hospital of Innsbruck from September 2008 to April 2013 after successful CPR. The presence of a neuroendocrine tumour, stroke, intracranial haemorrhage or trauma as cause of CA as well as life expectancy of less than 24 h as determined by the treating physicians were considered as exclusion criteria.

A sample size calculation was performed according to Moons et al. [16]. For each candidate predictor in the multivariate model 10 patients with poor neurological outcome were required. We assumed seven variables in our model leading to an event size of 70 patients. As about 50 % of the patients were expected to have poor neurological outcome based on clinical data from our unit, we aimed to have a total sample size of 140. Expecting 10 possible dropouts, we ended up with a total sample size of 150 patients.

Blood samples were drawn from the arterial catheter for the daily determination of SN and NSE starting at the day of CPR up to 7 days and assigned to the following time intervals: 0–24 h (day 0), 24–48 h (day 1), 48–72 h (day 2), 72–96 h (day 3), 96–120 h (day 4), 120–144 h (day 5) and 144–168 h (day 6).

SN samples were collected daily, centrifuged at 3000 rpm for 10 min and frozen at −80 °C for further analysis. SN measurements were performed with a radioimmunoassay according to Kirchmair et al. [17]. NSE levels were measured with an electrochemoluminescence assay (ECLIA, Roche, Mannheim, Germany). For safety issues the number of samples with haemolysis was documented for each patient.

Additionally lactate (Roche, Mannheim, Germany), as an established marker for circulatory shock, was measured after admission within 24 h.

Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were calculated at the day of ICU admission. CA data such as the rate of bystander resuscitation, time to ROSC and first monitored rhythm were collected from the emergency or heart alarm protocol according to the Utstein style [18]. Furthermore it was documented if patients underwent MTH using an intravascular cooling device targeting a core body temperature of 33 °C (measured in the urinary bladder) for 24 h. According to the guidelines MTH was routinely applied to comatose patients with an initially shockable rhythm that had received advanced life support within 15 min and showed a ROSC less than 60 min after collapse. After the change in the guidelines 2010 MTH was also applied to comatose patients with initially non-shockable rhythms if the event was observed and time to ROSC was less than 25 min [19].

Neurological assessment and outcome measure

Detailed neurologic examinations were performed in all patients who survived and were recorded primarily by a neurologist with expertise in neurocritical care after complete weaning off sedation [median day 5 (range 1–14) after CA for all study patients; median day 4 (range 1–13) for patients who did not undergo MTH and median day 6 (range 3–14) for patients treated with MTH], at

discharge from the ICU and at discharge from the hospital.

Neurological examination was performed in accordance to the American Academy of Neurology guidelines [10]. For patients who remained comatose after discontinuation of sedation, neurologic examination included testing for pupillary light responsiveness, corneal and oculocephalic reflexes, presence of gaze deviation, motor response to stimulation, and presence of myoclonus.

Further, neuroimaging findings, bilateral median nerve somatosensory evoked potentials (SSEP) recordings and bedside electroencephalograms (EEG) with standardized auditory and noxious stimulation were collected prospectively. Neurophysiologic examinations were performed by experienced technologists and interpreted by certified neurologists. Computed tomography (CT) scans were reviewed by dedicated neuroradiologists for the presence of global cerebral oedema and loss of differentiation between grey and white matter structures. In line with previous studies [20, 21], EEG patterns were grouped into malignant (e.g. non-reactive background, presence of burst-suppression, status epilepticus and alpha coma) and benign (any clear and reproducible change in amplitude or frequency of background on stimulation) categories. Importantly, all physicians involved in primary care and prognostication of study patients were blinded for SN analysis.

As primary outcome measure the Cerebral Performance Categories Scale (CPC) was recorded immediately before discharge either from hospital or a long-term care facility following a standardized protocol. The exact description of the five categories of CPC is provided in the Electronic Supplementary Material (ESM) [22, 23]. CPC 1 and 2 were considered as good neurological outcome, CPC 3–5 as poor neurological outcome. The study protocol was approved by the Ethics Committee of the Medical University of Innsbruck (protocol number UN3493 272/4.31). Written informed consent was obtained from next of kin or retrospectively from patients who recovered.

Medical decision making

Decisions about further medical treatment in terms of withdrawal or withholding of therapy were taken after completion of MTH, complete weaning off sedation and diagnostic procedures in an interdisciplinary conference of treating intensivists and neurologists. The decision was based on clinical, neurophysiological and imaging data as well as concomitant underlying diseases of the patient [6, 10]. NSE levels were available for the prognostication process. However, no decision was based only on this biomarker. The number of patients belonging to the following subgroups was documented: (1) withdrawal of therapy, (2) withholding of therapy, (3) maximal therapy.

Findings allowing withdrawal (1) or withholding (2) of therapy are listed below:

Brain death due to cerebral herniation, early severe myoclonus status and absence of bilateral median nerve SSEP allowed discontinuation of active treatment.

Withdrawal and withholding of therapy could also be done if patients stayed in persistent coma with negative SSEP or refractory status epilepticus after completion of MTH, complete weaning off sedation and diagnostic procedures.

Further, limiting of life-sustaining treatment could be considered in patients who retained SSEP, but did not improve in GCS for longer than 72 h after weaning off sedation.

Importantly, those patients had to be re-evaluated clinically and by means of EEG and neuroimaging.

Finally maximal therapy could be stopped because of ethical reasons (e.g. based on underlying diseases).

The medical consensus was discussed with the patient's family also considering the patient's presumed will. SN levels were not available for the treating physicians and did not influence this critical process in any case.

Statistical analysis

Categorical data are given as counts and percentages, continuous data as median and interquartile range. Normal distribution of continuous data was checked by the Kolmogorov–Smirnov test. As SN, NSE and most other variables were not normally distributed the Mann–Whitney *U* test and chi-squared test were used for univariate comparison of patients with good or poor neurological outcome.

For multivariable analysis a logistic regression model was used to assess the predictive importance of SN and NSE. All variables which showed statistical significance in the univariate analysis and were considered as being of clinical importance were included in the logistic regression model. The biomarkers were included in the model as continuous covariates. We visually inspected the relationship between NSE, SN and outcome and found log-linearity to be approximately fulfilled. There was no U-shaped distribution for both biomarkers.

The accuracy of SN and NSE levels to differentiate between good and poor outcome was evaluated by the receiver operating characteristic (ROC) analysis. Overall specific ROC curves were built for SN at 0–24, 24–48, 48–72 and 0–48 h and for NSE at 0–24, 24–48, 48–72 and 0–72 h. For analysis of any time period longer than 24 h the last observation carried forward (LOCF) method was used for missing value imputation, if necessary. Consequently, the latest measured values were chosen for all calculations.

The time intervals of 0–48 h for SN and 0–72 h for NSE were chosen according to the differing kinetics and

the discriminative power of the biomarkers assessed in ROC analysis for each 24 h period (requested cut-off AUC greater than 0.6).

The cut-off for determination of poor neurological outcome for SN was set at a specificity equal or greater than 95 %, as specificity is more important than sensitivity in a predictive model. The cut-off for NSE was set at 33 ng/ml on the basis of current guidelines [10].

Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were used to quantify improvement of predictability by combination of the two biomarkers. We applied the NRI with a three-category risk stratification [24] using the following risk categories: (1) less than 33 %, (2) 33–67 %, (3) at least 67 %. Reclassification indexes were calculated both univariately and multivariately.

Values with a p value less than 0.05 were considered as statistically significant. SPSS software (SPSS Inc., Chicago, Illinois, USA) and Stata 11 (StataCorp LP, College Station, Texas) were used to analyse data.

Results

Initially 152 patients were included in the study. Eighteen cases dropped out because of missing follow-up ($n = 4$), missing values ($n = 4$) or death due to non-neurological causes ($n = 10$). Finally a total of 134 patients remained for statistical analysis. (Table 1 ESM).

The median age was 64 (range 53–75) years, 35 patients were female. Ninety-one patients (67.9 %) had received bystander resuscitation. Seventeen patients (12.7 %) encountered an in-hospital CA. The initial rhythm was ventricular fibrillation in 77 patients (57 %), asystole in 34 patients (25 %), ventricular tachycardia in two patients (1 %) and pulseless electrical activity (PEA) in 17 patients (13 %). For four patients the ECG recordings of first monitored rhythm were not available (4 %).

Sixty-nine patients (51 %) had a poor outcome (CPC 3–5) at hospital discharge. Out of these, 12 patients ended up with poor outcome despite maximal therapy. In ten patients withholding of therapy and in 47 patients withdrawal of therapy were applied (Table 1 ESM).

Comparing patients with good and poor neurological outcome (CPC 3–5) a significant difference was observed in age, the rate of bystander-initiated CPR, the number of patients who had a time to ROSC greater than 20 min, the first monitored rhythm and the application of MTH ($p < 0.001$, respectively).

The SOFA and APACHE II score and lactate within 24 h after admission were also significantly higher in patients with poor neurological outcome ($p = 0.012$, less than 0.001 and 0.013 respectively) (Table 1).

In sixty patients (45 %) haemolysis interfered with NSE measurements at least in one sample.

SN and NSE kinetics

In the first 7 days after CA we observed a peak of SN in all patients within the first 24 h [0–24 h: 54 (28–87) fmol/ml]. The reference value of healthy volunteers is 9 fmol/ml [14].

During the following days SN values decreased to almost normal values [48–72 h: 31 (18–54) fmol/ml; 144–168 h: 15 (11–23) fmol/ml] (Fig. 1 ESM).

In contrast NSE peaked after 24 h [24–48 h: 34 (22–72) ng/ml; 48–72 h: 33 (18–92) ng/ml; 144–168 h: 18 (12–28) ng/ml]. According to the kinetics of both biomarkers, the first 72 h were considered the most significant period for determination and all further analyses were restricted to this time period (Fig. 2 ESM).

Univariate differences in patients with good and poor neurological outcome

Patients with poor neurological outcome (CPC 3–5) had significantly higher SN levels than patients with good neurological outcome (CPC 1–2) within the first 24 h [0–24 h: 75 (43–111) vs. 38 (23–68) fmol/ml; $p < 0.001$] and at 24–48 h after CPR [24–48 h: 45 (24–77) vs. 23 (16–39) fmol/ml; $p < 0.001$]. SN 0–48 h was also significantly higher in patients with poor neurological outcome [48 (29–89) vs. 26 (16–39) fmol/ml; $p < 0.001$] (Fig. 1a).

There were significant differences in NSE levels between patients with poor and good outcome in the first 24, at 24–48 and 48–72 h [0–24 h: 37 (26–55) vs. 30 (21–40) ng/ml, $p = 0.007$; 48–72 h: 81 (36–187) vs. 19 (17–23) ng/ml, $p < 0.001$].

NSE 0–72 h was also significantly higher in patients with poor neurological outcome [79 (27–193) vs. 20 (16–30) ng/ml, $p < 0.001$] (Fig. 1b).

Univariable prediction of poor neurological outcome by SN and NSE

In the first 48 h SN showed an AUC of 0.753 (95 % CI 0.665–0.841) with a sensitivity of 38 % at a specificity of 95 % to predict poor outcome. NSE in the first 72 h even had an AUC of 0.881 (95 % CI 0.815–0.946) with a sensitivity of 80 % at a specificity of 81 %, when setting the cut-off level at 33 ng/ml. ROC analysis for SN and NSE at each time interval is shown in Table 2.

Multivariable prediction of poor neurological outcome by SN and NSE

SN serum levels within the first 48 h and NSE within 72 h were significantly associated with poor outcome when adjusted for age, first monitored rhythm, time to ROSC, bystander-initiated CPR, MTH and lactate (logistic regression analysis) (Table 3).

When combining the two biomarkers in a logistic regression model an AUC of 0.925 (95 % CI 0.878–0.972) could be reached. Thus, by adding SN 0–48 h to NSE 0–72 h there was a significant improvement of 0.04 in c-statistics ($p = 0.03$) and a significant improvement of predictability of poor neurological outcome (IDI = 0.08 ± 0.03; $p = 0.001$). The NRI almost reached statistical significance (NRI = 0.15 ± 0.08; $p = 0.06$) (Fig. 2).

Using multivariate calculation of NRI and IDI, we still observed a nominal albeit not significant improvement in c-statistics from an AUC of 0.939 [0.897–0.980] to an AUC of 0.950 [0.912–0.988] when adding SN 0–48 h to NSE 0–72 h including covariates ($p = 0.275$). IDI remained statistically significant (IDI 0.04 ± 0.02; $p = 0.042$) and NRI was 0.09 ± 0.07 ($p = 0.164$).

When adding SN 0–48 h and NSE 0–72 h separately to a clinical model which included age, first monitored rhythm, time to ROSC, MTH, bystander-initiated CPR and lactate, we could observe an improvement in c-statistics in both cases, which was statistically significant only for NSE 0–72 h. IDI and NRI were significant for both markers. Detailed results are shown in Table 2 ESM.

Effect of MTH on SN

Fifty-nine patients (44 %) were treated with MTH. The majority (51 patients, 86 %) had an initially shockable rhythm whereas six patients (10 %) presented with asystole. Kinetics of SN was not influenced by the application of MTH. Overall both SN and NSE levels were lower in patients treated with MTH. However, when analysing the subgroups of patients with good or poor outcome separately, no significant differences in SN or NSE levels could be observed between patients treated with MTH and conventionally treated patients at any 24 h interval (Figs. 3 and 4 ESM).

Ancillary prognostic indicators

Results of clinical, neuroimaging and electrophysiological examinations are summarized in Table 3 ESM. For neuroimaging, global cerebral oedema as well as presence of decreased differentiation between grey and white matter structures was invariably associated with unfavourable outcome. Regarding electrophysiological testing, bilaterally absent N 20 response of median nerve SSEP remained a robust predictor of poor outcome. In addition, EEG patterns characterized as malignant were

Table 1 Comparison of patient characteristics and laboratory values

Patient characteristics	Good outcome ($n = 65$)	Poor outcome ($n = 69$)	p value
Age (years), median (IQR)	59 (50–70)	67 (58–76)	0.003
Female, *n* (%)	19 (29.2)	16 (23.2)	0.426
Bystander-initiated CPR, *n* (%)	52 (80.0)	39 (56.5)	0.004
Time to ROSC >20 min, *n* (%)	23 (35.4)	48 (69.6)	<0.001
Cardiac arrest out of hospital, *n* (%)	57 (87.7)	60 (86.9)	0.899
Mild therapeutic hypothermia, *n* (%)	37 (56.9)	22 (31.9)	0.004
Shockable first monitored rhythm (VFib + VTac)	50 (76.9)	29 (42.0)	<0.001
First monitored rhythm			
Ventricular fibrillation (VFib), *n* (%)	49 (75.4)	28 (40.6)	
Asystole, *n* (%)	7 (10.8)	27 (39.1)	
Pulseless electrical activity (PEA), *n* (%)	5 (7.7)	12 (17.4)	
Ventricular tachycardia (VTac), *n* (%)	1 (1.5)	1 (1.4)	
Unknown, *n* (%)	3 (4.6)	1 (1.4)	
Outcome at hospital discharge, *n* (%)			
CPC 1	51 (78.5)		
CPC 2	14 (21.5)		
CPC 3		3 (4.3)	
CPC 4		2 (2.9)	
CPC 5		64 (92.8)	
SOFA score on admission	10 (8–11)	11 (9–13)	0.012
APACHE II on admission	23 (20–26)	27 (23–31)	<0.001
Lactate 0–24 h (mg/dl)	41 (28–61)	59 (32–115)	0.013

CPC Cerebral Performance Categories scale, *CPC 1–2* good neurological outcome, *CPC 3–5* poor neurological outcome, *IQR* interquartile range, *VFib* ventricular fibrillation, *VTac* ventricular tachycardia, *SOFA* Sequential Organ Assessment score, *APACHE II* Acute Physiology and Chronic Health Evaluation II score

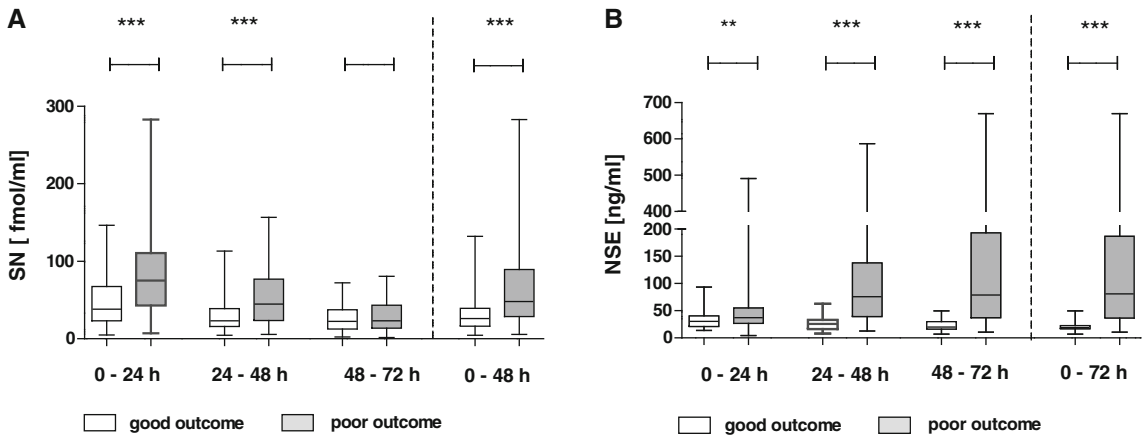

Fig. 1 **a** Secretoneurin (SN) serum levels of patients with good and poor outcome. SN levels [fmol/ml] in the first 72 h after CPR comparing patients with good and poor outcome; *n* (total) = 134; *n* (0–24 h) = 111; *n* (24–48 h) = 98; *n* (48–72 h) = 77; *n* (0–48 h) = 125. Graph shows median and range. ***$p < 0.001$. **b** Neuron-specific enolase (NSE) serum levels of patients with good and poor outcome. NSE levels [ng/ml] in the first 72 h after CPR comparing patients with good and poor outcome; *n* (total) = 134; *n* (0–24 h) = 119; *n* (24–48 h) = 87; *n* (48–72 h) = 57; *n* (0–48 h) = 128. Graph shows median and range. **$p < 0.01$, ***$p < 0.001$

incompatible with good neurological outcome. In contrast, reactive EEG background indicating a more benign pattern was associated with a more favourable outcome.

Discussion

This is the first study investigating SN as a possible biomarker for hypoxic brain injury after CPR. Serum levels of SN were peaking within 24 h after CPR showing a significant difference between patients with good and poor neurological outcome. SN was not affected by haemolysis or mild therapeutic hypothermia.

Upregulation of SN by hypoxia has already been demonstrated in several animal models as well as patients with stroke. The number of SN immunoreactive cells in the human brain showed a maximum at 3 days after onset of stroke, paralleled by increased SN serum levels peaking at the same day [25]. Immunoreactivity of SN was also found to be increased after transient forebrain ischemia in selected neuronal populations of the hippocampal formation and the cerebral cortex in mongolian gerbils. In this model the increase in SN immunoreactivity peaked already at 24 h. No measurements of SN serum levels, though, were performed in this experimental study [26].

The SN kinetics found in our study in patients after successful CPR showed a pattern of a rapid rise immediately after CPR with a steady decline over the following 72 h. The underlying mechanism currently remains a matter of speculation. One explanation could be that cerebral hypoxia directly increases expression of SN in the brain resulting in an enhanced release via the cerebrospinal fluid (CSF) into the blood. However, because the promoter of the SN precursor secretogranin II contains no hypoxia-responsive element, upregulation of SN under hypoxic conditions would be expected to be indirect and delayed compared to other factors directly

Table 2 Diagnostic accuracy of either SN or NSE at different time intervals to predict poor neurological outcome

	Cut-off	AUC	Sens	Spec ≥95 %	PPV	NPV
SN 0–24 h	121 fmol/ml	0.708 [0.611–0.806]	23	95	80	57
SN 24–48 h	58 fmol/ml	0.711 [0.606–0.816]	32	95	82	63
SN 48–72 h	54 fmol/ml	0.552 [0.417–0.687]	13	96	67	63
SN 0–48 h	65 fmol/ml	0.753 [0.665–0.841]	38	95	88	62
NSE 0–24 h	33 ng/ml	0.645 [0.546–0.743]	57	64	64	57
NSE 24–48 h	33 ng/ml	0.860 [0.778–0.943]	81	76	76	81
NSE 48–72 h	33 ng/ml	0.883 [0.787–0.979]	83	84	83	82
NSE 0–72 h	33 ng/ml	0.881 [0.815–0.946]	80	81	80	79

AUC area under the curve, *Sens* sensitivity, *Spec* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *SN* secretoneurin, *NSE* neuron-specific enolase

Table 3 SN and NSE predicting poor neurological outcome

	Odds ratio (95 % CI)	*p* value
NSE 0–72 h	1.110 (1.045–1.180)	0.001
SN 0–48 h	1.025 (1.001–1.049)	0.04

CI confidence interval, *ROSC* return of spontaneous circulation, *MTH* mild therapeutic hypothermia, *CPR* cardiopulmonary resuscitation, *NSE* neuron-specific enolase, *SN* secretoneurin

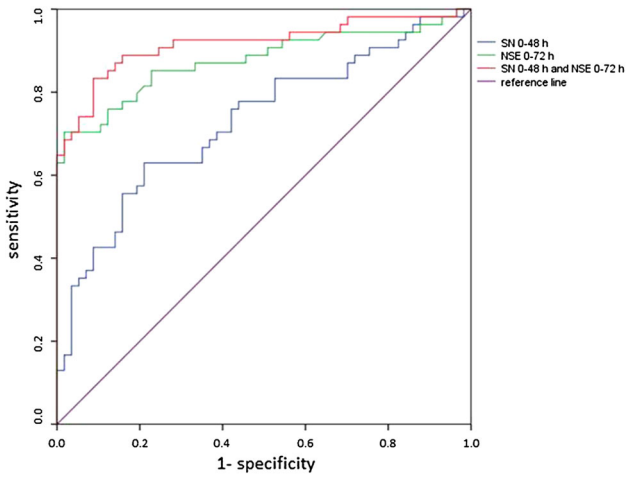

Fig. 2 ROC analysis for prediction of poor neurological outcome at 72 h after CPR. *SN* secretoneurin, *NSE* neuron-specific enolase

induced by hypoxia-inducible factor 1-α like vascular endothelial growth factor [27].

An alternative explanation could be a disruption of the blood brain barrier (BBB) following hypoxia during CA with a consecutive spill-over of SN from the CSF into peripheral blood. Under physiological conditions there is a concentration gradient of SN with levels in CSF exceeding serum levels by approximately 170 times [13]. With the exception of neuroendocrine tumours no extra cerebral production significantly altering SN serum levels is currently known [28]. By excluding patients with such a diagnosis we tried to avoid the possibility of increased SN levels resulting from anything else than hypoxic brain injury. The latter hypothesis may be supported by the time course of prediction of unfavourable neurological outcome by SN showing an AUC of at least 0.7 only within the first 48 h after CPR.

The performance of SN has to be appraised in the context of other neuronal tissue-specific biomarkers investigated after CPR:

S100B, an astroglial protein, has been implicated to be an early sensitive marker of hypoxic brain injury and short-term outcome after CA [12, 29–32]. Its predictive performance, however, may be impaired owing to extracerebral release of S100B after CPR, e.g. from adipocytes and chondrocytes [33]. Glial fibrillary acidic protein (GFAP), a structural protein of astrocytes, is elevated in serum after stroke, traumatic brain injury, subarachnoid haemorrhage and CA, but could not reliably predict poor outcome in patients treated with hypothermia after CA. Neither did brain-derived neurotrophic factor (BDNF) [30, 34].

The neurofilament light chain protein, a major structural element of the neuronal cytoskeleton, has been shown to be elevated in serum of patients with unfavourable long-term neurological outcome after CA from day 1 to day 7 [35].

With regard to plasma levels of selected tissue-specific microRNAs (miRNAs), it was demonstrated that brain-enriched miRNA-124 may be a biomarker for prediction of neurological prognosis between 24 and 48 h after CA [36]. The AUCs for predicting neurological outcome of all these markers ranged between 0.667 and 0.97 at various time points after CPR. However, all studies were rather small, the largest comprising 85 patients. Our study has at least twice the amount of patients. The ROCs obtained for SN attest to these markers being superior in several instances.

Unlike SN, NSE serum levels showed a slower increase after CPR in our study population peaking at 48–72 h. A similar behaviour of NSE was also observed in previous studies [37]. The later rise of NSE, as a marker of brain damage, compared to SN could be explained by the fact that the CSF/serum ratio of NSE is only 2.3 [38]. Consistent with other publications we showed an improvement in prediction of NSE after 24 h [31, 37]. The results of the PROPAC study lead to the conclusion that poor outcome in postanoxic coma can be reliably predicted as early as 24 h after CPR by means of NSE levels using a cut-off value of 33 ng/ml [10, 39]. However, several published data suggest considerably different NSE cut-off levels raising doubt about the predictive reliability of NSE [12, 31, 37]. The excellent AUC of NSE reported in our study has to be judged in the knowledge that in contrast to SN the treating physicians and neurologists were not blinded for NSE and consequently NSE kinetics may have influenced the decision-making process. This may also explain the slightly better effect of NSE 0–72 h when added to a clinical model including the covariates age, first monitored rhythm, time to ROSC, MTH, bystander-initiated CPR and lactate.

Additionally, reported shortcomings of NSE as an established marker must also be considered: First the determination of NSE is often impaired by even invisible haemolysis [40]. This was the case in any sample of 45 % of the patients in our study.

Secondly, a large body of evidence suggests that NSE levels are influenced by body temperature and that the predictive capability of NSE is reduced during MTH [41, 42]. Recommended cut-off levels for NSE at 72 h did not reliably predict poor neurological outcome in CA patients treated with MTH [11] resulting in high false positive rates [20]. We could not reliably confirm this observation

in our study, where both biomarkers—NSE and SN—appeared not to be significantly influenced by the application of MTH, although there appeared a trend to lower values for both biomarkers in the MTH group.

Although SN seems to have the capability to provide early prediction it is unlikely that clinically applicable prognostication will be accepted within the first 24 h. Consequently it was appealing for us to estimate the performance of SN in combination with NSE as a more established biomarker. Interestingly, we found that the combination of NSE and SN resulted in an AUC of greater than 0.9. Therefore it appears that combining two biomarkers with different kinetics, i.e. the early prediction by SN and the predictive capability of NSE at later time points, allows a more reliable prediction of outcome after the first 72 h. However, it has to be emphasized that even a combination of two biomarkers may only be used as "add-on" information but can never replace a multimodal approach to identify those patients with poor neurological outcome.

As the first report of SN as a marker of hypoxic brain injury associated with unfavourable prognosis after CPR, our study has several strengths and limitations:

It is one of the largest prospective observational trials investigating a biomarker performed in a population which was treated with state of the art care resulting in a good survival as reported by other high quality studies recently [6].

Furthermore, this is one of the few biomarker studies following a rigorous protocol defining withdrawal of therapy.

Among the limitations appears its design as a single centre study with the well-known influence of local case mix and treatment patterns.

Second, the mechanism of SN release remains speculative. Owing to frequent contraindication for CSF puncture in CA patients receiving anticoagulation, we did not perform measurements of NSE and SN in the CSF, which could have given us information about the concentration gradient between CSF and serum in the individual patients with hypoxic brain injury. Furthermore, we did not obtain a brain histology from patients who died to reliable confirm hypoxic brain injury. This, however, is a limitation which applies to nearly all published studies investigating outcome after CPR.

Third, only 7 % of our patients were classified as having died from a non-neurological cause. This number must be regarded under the condition that we excluded all patients who died within 24 h after admission to the ICU. A recent study retrospectively investigating the mortality after CA showed that post-CA shock was the leading cause of death in the first 3 days. Overall 269 of 1,125 patients admitted to the ICU (i.e. 24 %) died from post-CA shock, most of them in the first 2 days [43].

Fourth, the neurological outcome was determined with the CPC at hospital discharge, a marker that is predominantly used in resuscitation studies. We cannot rule out improvement of neurological function at later time points after final outcome assessment. However, assessment of CPC at hospital discharge includes the discharge from a secondary or tertiary hospital if prolonged rehabilitation or care was needed. Therefore a very long observation period was guaranteed.

Finally, patients were not randomized for the application of MTH. They were selected according to the guidelines described in the "Methods" section and therefore patients with a likely better outcome tended to receive MTH. To compensate for this selection bias we analysed each subpopulation—with good as well as poor outcome—separately for the possible influence of MTH on markers of neurological outcome and did not detect any significant difference.

In conclusion, our study indicates that SN might prove to be a promising early biomarker for the hypoxic brain injury after CPR. Within 24 h SN can predict neurological outcome earlier than NSE and is likely not influenced by the application of MTH. Considering the great interest in establishing a reliable early biomarker for severity of neurological damage after CPR for both prognostication and evaluation of upcoming therapeutic approaches as optimisation of post-resuscitation care, further studies on SN in the context of CA and CPR are warranted.

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Conflicts of interest The authors declare that they have no conflicts of interest.

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