Postanoxic electrographic status epilepticus and serum biomarkers of brain injury

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Abstract

Aim: To explore if electrographic status epilepticus (ESE) after cardiac arrest causes additional secondary brain injury reflected by serum levels of two novel biomarkers of brain injury: neurofilament light chain (NfL) originating from neurons and glial fibrillary acidic protein (GFAP) from glial cells.

Methods: Simplified continuous EEG (cEEG) and serum levels of NfL and GFAP, sampled at 24, 48 and 72 hours after cardiac arrest. All data were collected during the Target Temperature Management (TTM)-trial. Patients with and without ESE were matched for early predictors of poor neurological outcome.

Results: 128 patients had available biomarkers and cEEG. Twenty-six (20%) patients developed ESE, the majority (69%) within 24 hours. ESE was an independent predictor of elevated serum NfL ($p<0.001$) but not of serum GFAP ($p=0.16$) at 72 hours after cardiac arrest. Compared to a control group matched for early predictors of poor neurological outcome, patients who developed ESE had higher levels of serum NfL ($p=0.03$) and GFAP ($p=0.04$) at 72 hours after cardiac arrest.

Conclusion: ESE after cardiac arrest is associated with higher levels of serum NfL which may suggest increased secondary neuronal injury compared to matched patients without ESE but similar initial brain injury. Associations with GFAP reflecting glial injury are less clear. The study design cannot exclude imperfect matching or other mechanisms of secondary brain injury contributing to the higher levels of biomarkers of brain injury seen in the patients with ESE.

Keywords

Cardiac arrest, EEG, seizures, electrographic status epilepticus, biomarkers

List of abbreviations:

CPC	Cerebral Performance Category
EEG	Electroencephalography
cEEG	Continuous electroencephalography monitoring
GCS	Glascow Coma Scale
GFAP	Glial fibrillary acidic protein
ICU	Intensive care unit
NfL	Neurofilament light chain
NSE	Neuron-specific enolase
ROSC	Return of spontaneous circulation
SSEP	Somatosensory evoked potentials
TTM	Target tempertaure management
WLST	Withdrawal of life-sustaining treatments

Introduction

Electrographic status epilepticus (ESE) is found in up to one third of comatose survivors of cardiac arrest and is associated with a poor neurological outcome[1]. A minority of patients with ESE recover with a good neurological outcome[2]. In these patients ESE typically has an onset after rewarming and other neuroprognostic markers do not indicate extensive brain injury[3]. Further classification of ESE depending on discharge frequency appears not to affect the prognostic value of ESE after cardiac arrest[4].

After status epilepticus of other aetiologies, several biomarkers of neuronal injury are increased in cerebrospinal fluid[5], a sampling method often contraindicated after cardiac arrest

due to anticoagulation. Elevated levels of neuron-specific enolase (NSE) in serum has been found in status epilepticus of mixed pathophysiology[6].

Whether postanoxic ESE is simply a marker of severe encephalopathy or the cause of further secondary brain injury is controversial. Active treatment of seizures is recommended[7] since epileptic activity has the potential to increase the metabolic demand[8, 9] and thereby inflict additional brain injury. These recommendations are based on expert advice awaiting evidence from randomized trials[10].

Neurofilament light chain (NfL) is a novel biomarker of neuronal injury and a predictor of poor outcome after cardiac arrest[11]. After neuronal injury, serum NfL levels rise rapidly[12] and remain elevated for prolonged periods (weeks)[13]. Unlike NSE, NfL levels are not falsely elevated by hemolysis. Glial fibrillary acidic protein (GFAP) is a marker of glial cell injury with prognostic value after cardiac arrest[14]. Serum GFAP rises rapidly after cardiac arrest[15] and its half-life is long, up to 48 hours[13].

The aim of the present study was to explore if serum levels of NfL and GFAP can provide any evidence whether or not postanoxic ESE causes additional secondary brain injury. Our hypotheses were:

1. ESE is an independent predictor of serum levels of NfL and GFAP at 72 hours after cardiac arrest.
2. After onset of ESE, patients have higher levels of serum NfL and GFAP compared to a control group without ESE, matched for early predictors of poor neurological outcome.

Methods

This study uses data collected during the Target Temperature Management after Out-of-Hospital Cardiac Arrest trial (2010-2013)[16, 17]. Strict criteria for withdrawal of life-sustaining therapies (WLST) were applied. Sedation was mandatory during targeted temperature management (TTM) and continued after the intervention when indicated for medical reasons. Choice of sedative and antiepileptic drugs was not protocolized. Neurological outcome was assessed at 180-days using the cerebral performance category (CPC) scale. Serum biomarkers were sampled at 24, 48 and 72 hours after cardiac arrest, and batch analyzed as previously described[11, 14]. Laboratory methods used to measure GFAP (Banyan Biomarkers Inc) and NfL (Quanterix) have been described by the manufacturers.

Simplified cEEG was performed at six trial-sites using 4 electrodes (F3, P3, F4, P4), a reference in the Cz-position and ground in the Fz-position. All cEEG interpretations were performed by an EEG-specialist (EW) blinded to all clinical data[18]. The following patterns were considered to constitute ESE:

- Regularly appearing (=periodic or rhythmic) epileptiform discharges at ≥1Hz continuously (≥90%) appearing during a 30-minute-period.
- Unequivocal electrographic seizure activity with ≥10 second duration defined as generalized rhythmic epileptiform discharges ≥3Hz or clearly evolving discharges of any type reaching >4Hz, according to the EEG criteria of the American Clinical Neurophysiology Society[19].

Matched control group:

For patients with ESE, matched controls without ESE were identified by propensity score matching, using early (on admission) independent predictors of poor neurological outcome in

patients without missing data: age; first monitored rhythm; cardiac arrest at home; time to ROSC; treatment with adrenaline[20].

Statistics:

Continuous data are reported as median and interquartile range. Predictors of 72-hour serum levels were assessed by univariate and multivariate linear regression analysis with logistic transformation. Matched data were compared using Wilcoxon signed rank test. A two-sided p-value of <0.05 was considered significant. Expert statistical advice was sought for all analyses. Propensity score matching was performed using R with library Match It and optimal matching. For all other analyses IBM SPSS Statistics version 24 was used.

Results

At the six trial sites, 302 patients were included and 134/302 were monitored with cEEG (figure 1). In patients monitored with cEEG, clinical seizures were more common (40%) than in those who were not (26%), other patient characteristics were similar (supplementary table 1).

NfL and GFAP concentrations were measured in 128/134 patients; not all patients had biomarkers sampled at all time points (figure 1). ESE developed in 26/128 patients. Eighteen patients developed ESE within 24 hours, 6 between 24 and 48 hours, and 2 between 48 and 72 hours. One patient with ESE had a good long-term neurological outcome (CPC1 at 180 days). Compared to patients without ESE, patients with ESE had worse characteristics on admission, more often clinical seizures (85% vs 29%) and WLST (92% vs 29%) (table 1).

Neurofilament light chain

In a multivariate analysis, ESE was found to be an independent predictor of serum NfL levels at 72 hours ($p<0.001$) (supplementary table 3).

When compared to the matched control group (supplementary table 2), median serum levels of NfL were significantly higher in patients with ESE at 72 hours after cardiac arrest (4358 (IQR 1720-5364) vs. 142 (43-2661) pg/mL, $p=0.03$, figure 2). Although numerically higher, statistical significance was not reached at 24 hours (2227 (1341-4760) vs. 391 (38-3286) pg/mL, $p=0.30$) or 48 hours (3803 (1647-8035) vs. 130 (39-8144) pg/mL, $p=0.40$) (figure 2).

Glial fibrillary acidic protein

In multivariate analysis, ESE was not an independent predictor of serum GFAP at 72 hours ($p=0.16$, supplementary table 4).

When compared to the matched control group, serum levels of GFAP were significantly higher in patients with ESE at 72 hours after cardiac arrest (median 117 (IQR 71-305) vs 106 (31-965) pg/mL, $p=0.04$, figure 2). Levels were not significantly different at 24 hours (76 (53-145) vs 106 (31-965), $p=0.64$) or 48 hours (122 (82-229) vs 95 (19-723), $p=0.40$) (figure 2).

Discussion

ESE was an independent predictor of serum NfL levels at 72-hours after cardiac arrest. Patients with ESE had higher levels of serum NfL at 72-hours compared to a control group matched for early predictors of poor neurological outcome. These results may suggest additional neuronal injury in patients with ESE and are consistent with an earlier study where ESE was found to be an independent predictor of death[1].

ESE was not an independent predictor of serum GFAP at 72-hours, although GFAP levels in patients with ESE were higher at 72 hours compared with matched controls. The shorter half-life of GFAP compared with NfL (ref: https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/28717351/) may have attenuated differences between groups. However, the difference in results between the two biomarkers of brain injury may potentially also be explained by ESE predominantly causing injury to neurons as opposed to glial cells.

In an attempt to explore the question of causality and what comes first, the ESE or the secondary brain injury, a control group matched for early predictors of poor neurological outcome was identified among patients without ESE. The variables used for matching were cardiac arrest variables suggesting similar severity of the initial primary brain injury and therefore similar risk factors for developing secondary brain injury (due to reperfusion injury, fever, hyperglycemia, hypoperfusion, seizures, etc.). The biomarkers of brain injury NfL and GFAP were chosen due to their quick release and prolonged presence in serum after brain injury[13], making increasing levels in subsequent samples more likely to represent additional secondary injury compared to biomarkers of brain injury with shorter half-lives. At 72 hours after cardiac arrest, serum NfL and GFAP levels were significantly higher in patients with ESE compared to matched controls (levels at 24 and 48 hours did not reach significance), suggesting that ESE may cause additional secondary brain injury.

Strengths and limitations

Our results are strengthened by the multicentre study design, prospective data collection and strict criteria for WLST. We used available data to perform matching but acknowledge that matching based on pre-hospital data is imperfect by default. Conclusions are hampered by

missing data. Due to the explorative nature and small number of data correlations of repeated measurements were not accounted for. The TTM-trial was not primarily designed to investigate ESE, *e.g.*, serum sampling was not timed according to ESE onset. Additionally, the study design cannot exclude other mechanisms of secondary brain injury as a cause for the higher levels of biomarkers of brain injury seen in ESE. The attempt to match the groups based on cardiac arrest characteristics may be too blunt, creating groups with important differences in severity in brain injury. Therefore, our data should be regarded as hypothesis-generating and need to be tested prospectively. Some patients received antiepileptic drugs due to clinical and/or electrographic seizures. Due to variability in treatment with antiepileptics, its effect of serum biomarkers could not be assessed.

Conclusions

After cardiac arrest, ESE is associated with higher levels of serum NfL which may indicate a potential secondary neuronal injury caused by ESE. Associations with GFAP, a marker of astrocytic activation/injury, are less clear.

Conflicts of interest

Anna Lybeck, Tobias Cronberg, Niklas Nielsen, Malin Rundgren and Erik Westhall have none to declare. Hans Friberg is a scientific advisor for QuickCool (Lund, Sweden). Henrik Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

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Ethics and Patient Consent

Sweden: Regional Ethical Review Board Lund, Protocol 2009/6 Dnr 2009/324 (TTM-Trial). Denmark: De vitenskabsetiske Komiteer i Region Hovedstaden, H-1-2010-059. The Netherlands: Medisch Etische Toetsingscommissie MEC 10/107 # 10.17.0921. In accordance with national requirements, written informed consent was waived, delayed, or obtained from a legal surrogate, and was obtained from each patient who regained mental capacity.

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collection, analysis or interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

Table 1: Characteristics of patients with ESE and without ESE among cEEG monitored patients with available biomarkers. Data presented as median (IQR), n (%).

	ESE n=26	No ESE n=102
Sex, female	5 (19%)	20 (20%)
Cardiac arrest at home	17 (65%)	55 (54%)
Shockable rhythm	15 (58%)	83 (83%)
Treatment with adrenaline	23 (89%)	67 (66%)
ROSC, minutes, median (IQR)	34 (23-46)	25 (16-35)
GCS motor score 1 on admission	22 (92%) n=24	51 (58%) n=88
Corneal and pupillary reflexes absent on admission	10 (43%) n=23	32 (38%) n=84
TTM33	14 (54%)	50 (49%)
Clinical seizures	22 (85%)	31 (29%)
Antiepileptic therapy*		
monotherapy	10 (39%)	15 (15%)
combination	6 (23%)	6 (6%)
SSEP bilaterally absent	7 (27%)	14 (14%)
Poor neurological outcome (CPC3-5)	25 (96%)	43 (42%)
WLST	24 (92%)	30 (29%)

*antiepileptic treatment started due to electrographic seizures and/or clinical seizures

Legends to figures

Figure 1: Study flow-chart of patients at the six sites with cEEG monitoring. *Serum biomarkers were sampled at 24, 48 and 72 hours. Samples from all three timepoints were not available for all 128 patients. ESE= electrographic status epilepticus.

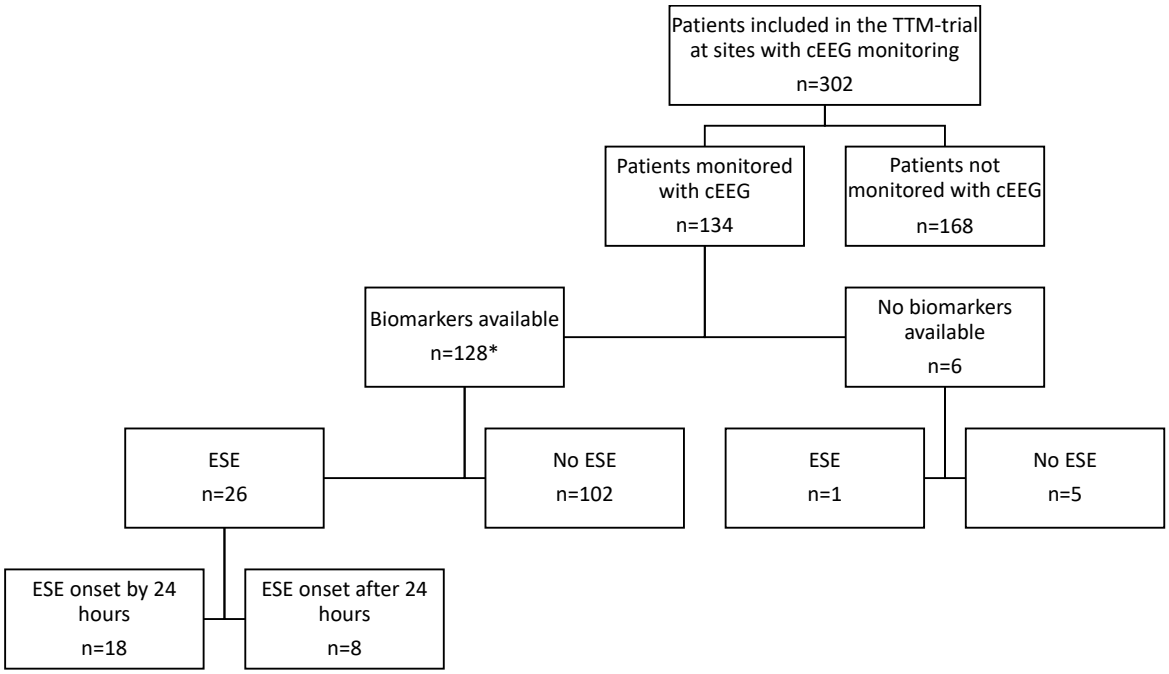
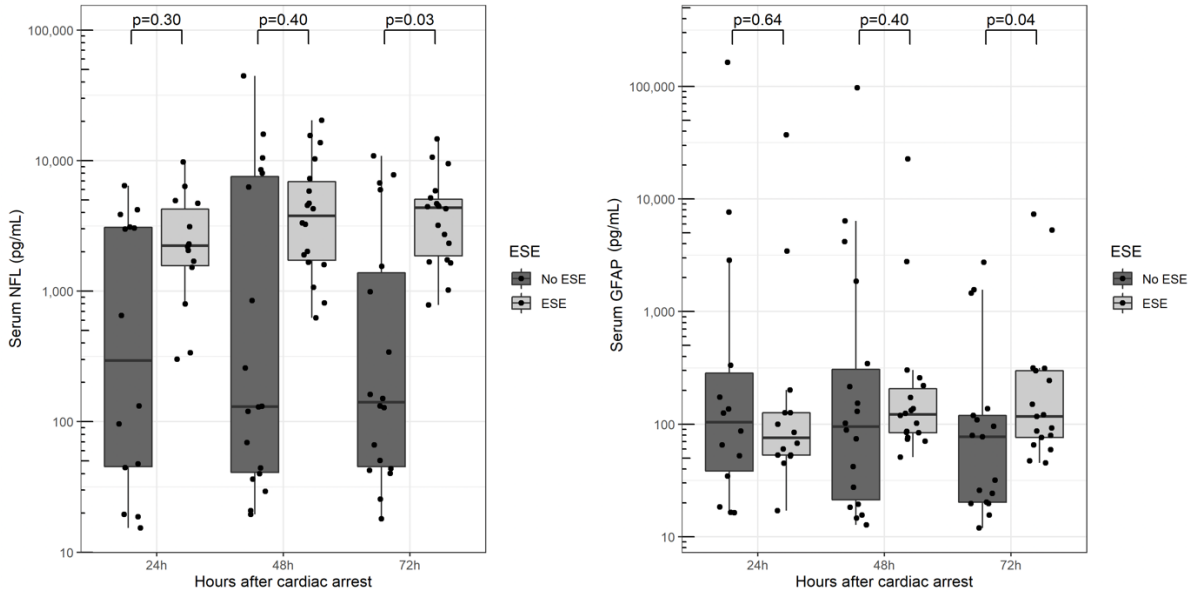


Figure 2: Biomarkers of brain injury in patients with ESE compared to matched controls. Serum levels of NfL and GFAP (pg/mL) at 24, 48 and 72 hours after cardiac arrest. Patients who developed ESE (and their matched controls) were included in the analysis only after onset of ESE and when biomarkers were available (for both case and matched control). The number of matched pairs available at 24, 48 and 72 hours were 14, 18, 18 for NfL and 13, 18, 17 for GFAP. The lower boundary of the boxes indicate the 25th percentile; horizontal line within the box, median; higher boundary of the box, 75th percentile; error bars, 90th and 10th percentiles.



SUPPLEMENT

Supplementary table 1: Characteristics of patients monitored with cEEG vs patients with no cEEG monitoring.

Variable	**cEEG (n=134)**	**No cEEG (n=168)**
Age (years)	66 (56-72)	63 (53-69)
Sex (female)	28 (21%)	21 (13%)
CA at home	75 (55%)	95 (57%)
Shockable rhythm	101 (75%)	139 (83%)
Treatment with adrenaline	93 (69%)	128 (76%)
Time to ROSC (minutes)	25 (17-38)	24 (14-35)
GCS motor score 1 on admission	73 (63%) n=115	119 (88%) n=136
Corneal and pupillary reflexes absent on admission	38 (34%) n=112	8 (5%) n=150
TTM33	68 (51%)	85 (51%)
Clinical seizures	53 (40%)	44 (26%)
SSEP bilaterally absent	21 (13%)	10 (6%)
Poor neurological outcome (CPC3-5)	72 (54%)	72 (43%)
WLST	57 (43%)	50 (39%)

Median (IQR), n (%).

Supplementary table 2: Comparison of the matched control group vs cases with ESE

Variable	**Matched controls n=26**	**ESE n=26**
*Age (years)	69 (66-78)	72 (65-81)
*Sex (female)	6 (23%)	5 (19%)
*CA at home	16 (62%)	17 (65%)
*Shockable rhythm	14 (54%)	15(58%)
*Treatment with adrenaline	22 (85%)	23 (89%)
*Time to ROSC (minutes)	35 (20-44)	34 (23-46)
GCS motor score 1 on admission	17 (68%) (n=25)	21 (91%) (n=23)
Corneal and pupillary reflexes absent on admission	11 (46%) (n=24)	10 (43%) (n=23)
TTM33	17 (65%)	14 (54%)
Clinical seizures	11 (42%)	22 (85%)
Antiepileptic therapy		
monotherapy	9 (35%)	10 (39%)
combination	2 (8%)	6 (23%)
SSEP performed	8 (31%)	20 (77%)
SSEP bilaterally absent	7 (26%)	7 (26%)
Poor neurological outcome (CPC3-5)	15 (58%)	25 (96%)
WLST	12 (46%)	24 (96%)

Median (IQR), n (%). *variables used for matching due to no missing data.

Supplementary table 3: Predictors of serum NfL at 72 hours. Serum NfL at 72 hours was available for 105/128 patients.

Variable (n=105)	**Univariate analysis**		**Multivariate analysis**	
	exp (B) (95%CI)	**p**	**exp (B) (95%CI)**	**P**
Age	1.02 (0.98-1.06)	0.29	0.99 (0.96-1.03)	0.73
Sex (female) (20/105)	1.12 (0.40-3.14)	0.83	1.33 (0.60-2.95)	0.48
Cardiac arrest at home (54/105)	2.40 (1.09-5.30)	0.03	1.28 (0.66-2.46)	0.46
Non-shockable rhythm (15/103)	5.3 (1.76-15.94)	<0.01	2.76 (1.08-7.04)	0.03
Time to ROSC	1.03 (1.02-1.05)	<0.001	1.02 (1.00-1.03)	0.02
Dose of adrenaline	1.32 (1.15-1.52)	<0.001	1.15 (1.00-1.32)	0.05
Bilaterally absent pupillary and corneal reflexes on admission (25/103)	5.85 (2.40-14.28)	<0.001	2.84 (1.34-5.98)	<0.01
GCS motor score on admission (n=94)	0.52 (0.29-0.70)	<0.001	0.77 (0.59-1.01)	0.05
ESE (19/105)	18.57 (7.65-45.11)	<0.001	7.20 (2.93-17.65)	<0.001

Supplementary table 4: Predictors of serum GFAP at 72 hours. Serum GFAP at 72 hours was available for 105/128 patients.

Variable (n=105)	**Univariate analysis**		**Multivariate analysis**	
	exp (B) (95%CI)	**p**	**exp (B) (95%CI)**	**p**
Age (105/105)	1.04 (1.01-1.07)	<0.01	1.04 (1.00-1.07)	0.03
Sex (female) (19/105)	2.07 (0.91-4.74)	0.08	2.87 (1.30-6.33)	0.01
Cardiac arrest at home (54/105)	0.21 (0.63-2.30)	0.56	0.80 (0.42-1.55)	0.51
Non-shockable rhythm (14/103)	5.01 (2.06-12.21)	<0.001	2.41 (0.92-6.31)	0.07
Time to ROSC	1.03 (1.01-1.04)	0.01	1.02 (1.01-1.04)	0.01
Dose of adrenaline	1.19 (1.06-1.33)	<0.01	1.07 (0.93-1.22)	0.34
Bilaterally absent pupillary and corneal reflexes on admission (25/103)	3.38 (1.55-7.35)	<0.01	1.87 (0.90-3.89)	0.09
GCS motor score on admission (94/105)	0.70 (0.55-0.89)	<0.01	0.73 (0.56-0.96)	0.02
ESE (18/105)	3.35 (1.47-7.64)	<0.01	1.88 (0.76-4.60)	0.16

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