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Cardiac arrest and therapeutic hypothermia: Prognosis and outcome

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LUND UNIVERSITY Faculty of Medicine

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List of publications

The present thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. Rundgren M, Rosén I, Friberg H. Amplitude integrated EEG (aEEG) predicts neurological outcome in patients after cardiac arrest and induced hypothermia. Intensive Care Medicine 2006; 32: 836-42.

II. Rundgren M, Karlsson T, Nielsen N, Cronberg T, Johnsson P, Friberg H. Neuron specific enolase and S-100B as predictors of outcome after cardiac arrest and induced hypothermia. Resuscitation 2009; 80: 784-9.

III. Cronberg T, Lilja G, Rundgren M, Friberg H, Widner H. Long-term neurological outcome after cardiac arrest and therapeutic hypothermia. Resuscitation 2009; 80: 1119-23.

IV. Rundgren M, Westhall E, Cronberg T, Rosén I, Friberg H. Continuous amplitude integrated EEG predicts outcome in hypothermia treated cardiac arrest patients. Manuscript.

V. Cronberg T, Rundgren M, Westhall E, Englund E, Siemund R, Rosén I, Widner H, Friberg H. Therapeutic hypothermia after cardiac arrest: What characterizes the patient remaining in coma? Manuscript.

Abbreviations

ADL:	Activities of daily life
aEEG	Amplitude integrated electroencephalogram
ATP:	Adenosine triphosphate
BIS:	Bispectral index
CA:	Cardiac arrest
CBF:	Cerebral blood flow
CI:	Confidence interval
CPC:	Cerebral performance categories
CPP:	Coronary perfusion pressure
CPR:	Cardiopulmonary resuscitation
CT:	Computer tomography
EEG:	Electroencephalogram
ESE:	Electrographic status epilepticus
GCS:	Glasgow coma scale
IABP:	Intraaortic balloon pump
IHCA:	In-hospital cardiac arrest
ICU:	Intensive care unit
MAP:	Mean arterial pressure
MRI:	Magnetic resonance imaging
N20:	Early cortical peak
N70:	Late cortical peak
NSE:	Neuron specific enolase
OHCA:	Out-of-hospital cardiac arrest
RCT:	Randomised controlled trial
ROSC:	Return of spontaneous circulation
SB:	Suppression burst
SSEP:	Somatosensory evoked potentials (median nerve)
S-100B:	Protein marker of astrocyte damage
TH:	Therapeutic hypothermia
VF:	Ventricular fibrillation
VT:	Ventricular tachycardia

Introduction

Cardiac arrest (CA) is a common cause of death in the western world. It is a medical emergency that usually takes place outside the hospital, and in Sweden the yearly incidence is 70-110/100,000.¹ The time from CA to return of spontaneous circulation (ROSC) has a strong influence on long-term outcome.²⁻⁴ Therefore, lay rescuers can make a profound difference for the affected individual by acting correctly, i.e., call for ambulance and start chest compressions.⁵

In CA, all organs are affected by ischemia. The ischemia and the subsequent reperfusion result in a generalised inflammatory response.⁶ The brain is especially vulnerable to ischemia/reperfusion and its secondary effects, and irreversible changes start to take place within minutes.⁷ This is of major clinical importance, reflected by the fact that a high percentage of patients with CA that regain ROSC survive with, or succumb to, irreversible brain damage.⁸ Therefore reliable methods for assessment of the neurological prognosis are of outmost importance.

For the last 40 years, ways of ameliorating brain injury caused by ischemia and reperfusion have been evaluated. In the last decade, therapeutic hypothermia (TH) has emerged as a feasible option for unconscious CA victims.^{9, 10} TH demands sedation and intermittent paralysis, obscuring any clinical neurological status during the first 36-48 hours after CA.¹¹ With an increased use of TH, a need for objective prognostic markers has become even more urgent.

The neurological examination is the basis for prognostication. The use of neurophysiological methods (EEG and SSEP) and biochemical markers (including S-100B and NSE) in CA has been considered as complement to neurological examination for a number of years.¹²⁻¹⁷ However, at the start of the present study (i.e., in 2003), while S-100B and NSE had been employed at CA, this was not in the context of TH. aEEG had been used in asphyctic neonates,¹⁸ but not in patients after CA and TH.

The aim of the present thesis was to develop and evaluate clinically relevant methods for early prognostication of neurological outcome in patients receiving TH following CA, focusing on aEEG, NSE, and S-100B. Increasing our ability to prognosticate neurological outcome during the intensive care period is important in order to make information to relatives more reliable and aid in decision-making regarding level of care.

Background

Cardiac arrest

Aetiology

The major cause of CA is myocardial ischemia and infarction, which may trigger VT/VF. Such malignant arrhythmias may occur in the acute phase of ischemia, but typically also at reperfusion.¹⁹ During the last decades, a decrease in VF as the initially registered rhythm in CA has been observed.^{20, 21} VT/VF may also occur as primary arrhythmias, and in some cases they may have a hereditary cause.²² Finally, there are non-cardiac causes of CA, including pulmonary embolism, hypovolemia, asphyxia, drowning, electrolyte disturbances, and suicide attempts, e.g., hanging and drug overdoses with secondary hypoxia or arrhythmias.²³

Cardiopulmonary resuscitation history

The external chest compression technique was introduced in 1960.²⁴ Among the initial patients studied, a majority of cases were peri-operative CAs. Fortunately, anaesthesia techniques and patient monitoring have developed since then, and unexpected CA is now unusual in the operating theatre. A combination of closed chest compression and ventilation, i.e., "CPR", was presented later the same year. Already at this point, hypothermia was suggested as post-resuscitation support for unconscious victims.²⁵ Moderate hypothermia (28-30°C) was recommended, but this temperature range was associated with adverse events involving the circulation and coagulation systems, and therefore TH was abandoned.

Treatment

The success-rate of CA treatment, measured as percentage of patients obtaining ROSC, is greater with a shorter time from CA to defibrillation in cases with VF or pulseless VT.²⁶ It has also been shown in animal experiments that the efficiency of external cardiac compressions is of importance to increase the CPP and to optimize the myocardial response to defibrillation.²⁷ In addition to chest-compressions and defibrillation, medical treatment (e.g., epinephrine, atropine, and amiodarone) is used. However, whereas a small RCT has suggested benefit of a combination of adrenaline, vasopressin, and metylprednisolon in association with resuscitation,²⁸ no large scale RCTs have shown long term survival benefit of medications administered during CPR.²⁹⁻³¹

Current recommendations

Cardiopulmonary resuscitation guidelines are revised continuously. In the latest comprehensive revision (i.e., from 2005), emphasis was put on optimising CPR to allow only minimal interruptions in chest compressions to optimize myocardial perfusion. Recommendations regarding post-resuscitation care were addressed and the ILCOR statement of 2003,³² recommending TH for 12-24 hours at 33°C for unconscious OHCA with VT/VF patients, and possibly for other initial rhythms and IHCA, was consolidated.³³ New guidelines are expected in November 2010.

Global cerebral ischemia

Physiology of cerebral ischemia

A circulatory arrest results in failure of ATP production. A reduction of ATP levels by 70% produces a sudden loss of membrane potential, i.e., anoxic depolarisation.³⁴ The lack of ATP also induces an increase in intracellular Ca^{2+} , reflecting intracellular release and influx from the extracellular space.³⁵ Ca^{2+} is an intracellular second messenger and the increase stimulates release of neuro-transmitters, including glutamate. Also, lack of ATP inhibits reuptake of glutamate and the extracellular concentration of this transmitter (and of other transmitter substances) increases.³⁶ This increase is the basis for the *exotoxicity hypothesis* of cerebral ischemic damage.

Physiology of initial reperfusion

Neuronal damage occurs during the ischemic period, but also during reperfusion that triggers a secondary cascade of events. Na^+/K^+ gradients are normally restored prior to restoration of ATP, reflecting that the initial ATP production is used to restore the membrane potentials.³⁷ This process is slowed down when ischemia times exceeds 15 minutes.³⁸ Furthermore, during the initial phase of reperfusion, tissue toxic free radicals are generated³⁹ and mitochondrial functions may be impaired.⁴⁰ A secondary deterioration, lasting several days, ensues.⁴¹ The temporal course of this process coincides with a secondary increase in intracellular and intra-mitochondrial Ca²⁺ levels.

Cerebral perfusion and cerebral blood flow at reperfusion

With stable ROSC, a short time-period (minutes) of increased cerebral blood flow ensues.⁴² The duration of the hyperaemia increases with increasing length of the ischemia.⁴³ After the hyperaemic period, CBF is reduced to subnormal levels. This hypoperfusion slowly recovers over the first 6-12 hours after the CA, but it may also be more sustained.⁴⁴⁻⁴⁶

The blood flow at reperfusion has an inhomogeneous distribution with areas of no "re-flow" increasing with longer CA duration.⁴⁷ During early reperfusion CO_2 reactivity is impaired,⁴⁸ and global CBF is proportional to the systemic blood pressure.

Clinically, the situation after ROSC is complex. There is evidence for an affected cerebral auto-regulation.⁴⁹ In addition, the cerebral circulation may be affected by vascular disease, which is common in this group of patients (Englund, personal communication). The cardiac output after ROSC may be reduced by myocardial stunning secondary to the CA or ongoing myocardial ischemia.⁵⁰ The combination of reduced cardiac output and reduced MAP may affect the global cerebral circulation, increasing the risk of an inadequate perfusion.

Recovery or cell death?

If the acute lack of perfusion is brief, the membrane potential and energy metabolism in the brain is restored within minutes. When ROSC is attained after an ischemic injury of "intermediate" duration, a delayed (apoptotic) cell death may take place over the first couple of days.⁵¹ In the case of a global ischemia of long duration, or lack of ROSC, brain necrosis will occur with cellular swelling and disintegration of cellular membranes.⁵²

The apoptotic cell death is energy demanding and includes an altered balance of apoptosis inhibitor and promoter proteins,⁵³ release of mitochondrial cytochromec, and induction of caspases.⁵⁴ Evidence of delayed neuronal death has been found in hippocampus in humans.⁵⁵

Necrotic and apoptotic cell death patterns can coexist within the injured brain.⁵⁶ Interfering with the sequence of events leading to apoptosis may be a way to ameliorate acute ischemic injury, and forms the theoretical basis for the use of post-ischemic interventions. In human CA, interventions targeting the reperfusion injury are particularly relevant, since the CA occurs unexpectedly and treatment prior to the event is impossible. When targeting the later phases of the reperfusion injury therapeutically, there is a time window between the CA and the secondary deterioration that allows for intervention.

Selective vulnerability of the brain

In the brain, there is a selective vulnerability to ischemic insults. The CA1-region of the hippocampus, parts of the basal ganglia, certain layers of the neocortex, and cerebellar purkinje-cells are known to be sensitive to ischemic insults,⁵⁷ and the fraction of affected neurons increases with increasing ischemia times.⁵⁸ The most vulnerable neuronal groups are involved in memory functions (i.e., the hippocampus, the amygdala, and the caudate nucleus), and defects in these functions are

prominent in patients recovering from CA.⁵⁹ Other neurons, for instance in the medulla, pons, and midbrain, are more resistant to ischemic insults and reperfusion.⁷

Trials of pharmacological interventions

Pharmacological treatments potentially relevant to neurological damage secondary to CA have shown some promise in controlled animal studies. These treatments address single injury mechanisms: e.g., thiopental (for reduction of cerebral metabolism) and lidoflazine (a calcium entry-blocker). Both have reached clinical trials, but none have shown convincing benefit in a clinical setting.^{60, 61} There may be several reasons for the difficulties in translating animal data into the clinical context. First, the inter-individual variability of human CA is greater than in controlled situations involving animals of the same age, breed, and genetic background. Secondly, the unexpected CA in humans is inherently different to that of an experimental situation since ischemia times are less well controlled, quality of resuscitation varies, and in-hospital treatments differ.⁶²⁻⁶⁴

Hypothermia mechanisms

Cerebral metabolism is temperature dependent. Reflecting this, oxygen consumption in the rat brain decreases with about 5% per degree reduction in body temperature.⁶⁵ When hypothermia is present at the start of cerebral ischemia, the time to anoxic depolarisation is prolonged.⁶⁶ In global ischemia, the rate of ATP depletion is delayed in hypothermia, but reaches the same levels as in normothermia after 20 minutes.⁶⁷ The decrease in metabolism (20-25%) at 32-33°C is small in relation to the protective effects, suggesting a role also for other mechanisms.⁶⁸

Non-metabolic mechanisms include suppression of exotoxines and reactive oxygen species. Accordingly, intraischemic hypothermia may reduce glutamate release⁶⁹ and free radical production.⁷⁰ However, these mechanisms are predominant during ischemia and in the early reperfusion period, and may not account for the effect of "delayed cooling". Delayed cooling may be effective by attenuating events triggering delayed cell death, i.e., down-regulation of pro-inflammatory and pro-apoptotic genes, up-regulation of trophic genes, and anti-apoptotic effects involving cytochrome-c release from mitochondia and caspase activation.⁶⁸ It has been shown that delayed cooling reduces the secondary energy failure.⁷¹

Global ischemia and hypothermia in animal studies

In the late 80s, it was reported that intraischemic mild hypothermia (34°C) improved the neurological outcome in dogs subjected to CA.⁷² Protection was more pronounced than expected from a reduction of metabolism alone, considering the modest reduction in temperature. In addition, in studies focusing on temperature control, it was shown that a graded temperature increase (33-39°C) resulted in a worsened histopathological outcome despite similarly depleted ATP levels.⁶⁷ The protective effect of hypothermia was shown to be long lasting.⁷³

Whether or not induction of hypothermia instituted after a global ischemia would produce a cerebral protective effect was then studied in several species.⁷⁴⁻⁷⁶ In dogs, induction of hypothermia after 20 minutes of resuscitation for VF, during continued resuscitation and hypothermia at 34 and 27°C for 12 hours, improved 96 hour functional and histological outcome (*c.f.* normothermia).⁷⁶ The impact of the depth of hypothermia was also addressed in dogs subjected to 12.5 minutes of VF and mild (33°C) and moderate (30°C), but not deep (15°C), hypothermia improved functional outcome.⁷⁷ With short-term hypothermia, i.e., three hours (in a post-ischemia rat model), the neuroprotective effect tapered off during two months following the ischemic insult.⁷⁸

Colbourne *et al.* assessed six months morphological outcome in the gerbril hippocampus, and found a reduction in protection from 70 to 12% when delaying a 24 hour cooling period from one to four hours after the insult.⁷⁹ In the same setting, an increased duration of cooling from 24 to 48 hours improved the morphological outcome even if the delay of start of cooling increased to six hours.⁸⁰

In conclusion, the effect of hypothermia induced after global ischemia in animal studies is better if the post-ischemic delay to hypothermia is shortened and if the treatment is prolonged.

Clinical applications of hypothermia

Cardiac arrest

Based on reproducible effects in animals, feasibility studies were undertaken during the 90s to test the applicability of TH in human CA. The aim was fast cooling to 33-34°C for 12-48 hours. Most studies used external approaches, i.e., ice-packs, fans, forced air cooling, cooling blankets, or helmets,⁸¹⁻⁸⁴ but extremely invasive methods including the combination of IABP and cardiopulmonary bypass

were also employed.⁸⁵ All studies suggested that TH was feasible, but for the external cooling studies there were problems in achieving fast cooling. However, the amount of arrhythmias, infections, and coagulopathies were considered acceptable. In these small studies, overall outcome was better than or equal to historical controls.

In 2002, two RCTs, including OHCA patients of presumed cardiac origin and with VT/VF as presenting rhythm, were published:

- i. In a multi-centre study, 273 individuals were examined.⁹ Patients received TH at 33°C for 24 hours and TH was compared to conventional treatment. A favourable six months neurological outcome was reported for 75/136 (55%) of TH patients vs. 54/137 (39%) in the conventionally treated group.
- ii. In a multi-centre study by Bernard *et al.*, involving 77 patients with CA, TH was given for twelve hours. 21/43 (49%) of the TH individuals could be discharged home or to a rehabilitation facility vs. 9/34 (26%) in the group not receiving TH.¹⁰

The differences between TH and conventional treatment were statistically significant in both studies. However, the studies have been criticised due to incomplete temperature control in the non-treatment group⁹ and the randomisation procedure.¹⁰ Furthermore, they both covered adult OHCA patients with VT/VF as initial rhythm and a presumed cardiac cause, and excluded patients with unstable circulation. In subsequent implementation studies and registry reports, the extension of TH outside the originally study groups is evident e.g., inclusion of patients with non-VF/VF rhythms and shock.^{2, 3, 86} Additional RCTs in adult patients are ongoing (Wolfrum *et al.*, NCT00475431) or planned (Nielsen *et al.*, NCT01020916).

Neonatal asphyxia

Neonatal asphyxia is a situation where the neonate is exposed to a hypoxic insult usually caused by inadequate placental blood-flow. The depth and duration of neonatal asphyxia may vary substantially, but affected neonates show signs of anaerobic metabolism (lactate increase, pH decrease) in combination with specific clinical signs (low Apgar-scores, cerebral irritability/seizures). Thus, the insult to the brain is similar to that of CA with ischemia/anoxia and reperfusion injury. Asphyxia, if severe enough, is associated with high mortality and morbidity in the form of neurological sequels. Based on animal experiments,⁸⁷ TH (33.5-35.0°C for 72 hours) has emerged as a clinically feasible option to increase survival and to ameliorate neurological sequels following asphyxia in the neonate.⁸⁸⁻⁹⁰

Other indications

In cardiac surgery, hypothermia has been used as a neuroprotective measure since the 50s. The setting is different compared to CA, since pre- as well as intraischemic hypothermia can be employed. Hypothermia has also been clinically evaluated in traumatic brain injury, and stroke. However, the associated pathophysiology with a vulnerable penumbra zone, differ substantially from the global ischemia at CA.⁹¹ No convincing benefit has been shown in these latter situations.^{92, 93}

Neurological examination

The natural course of recovery

Neurological recovery over the first year following CA has prospectively been examined by Jørgensen *et al.*^{59, 94, 95} Initial recovery comprises a return of cranial nerve reflexes and spontaneous breathing. Thereafter, return of extension pattern, defensive movement, and eventually consciousness with a gradual return of speech, motor functions, orientation, and memory functions. The sequence of recovery follows a distinct pattern, but it can be halted and the time-course varies.

Clinical neurological examination

A clinical examination forms the basis for the evaluation of the neurological prognosis in patients in coma after CA. The examination focuses on the level of consciousness and function of the brainstem. From clinical studies and metanalyses^{13, 96-98}, signs with a high specificity for a poor outcome (death or vegetative state) have been suggested.

These clinical signs are:

- i. Bilateral absence of pupillary reflex to light.^{13, 96-98}
- ii. Bilateral absence of corneal reflexes.^{97, 90}
- iii. No motor response or an extension pattern to pain stimuli.^{13,96-98}

The prognostic accuracy increases with increasing time from the CA. In nonhypothermia treated CA patients, these clinical tests are considered accurate three days after the CA. A poor test result may be seen in <20% (no pupillary reactions day 3) to 48 % (at best an extension pattern to pain stimulation) in patients remaining unconcious,¹³ indicating that a substantial number of patients will remain unconscious despite not showing clinical signs suggesting a poor outcome. These signs were tested prospectively in the PROPAC study. The study concluded that the neurophysiological and biochemical tests were superior to clinical tests.¹⁴

Due to the temporal development of improvement after global ischemic brain injury, the clinical neurological examination is unreliable in predicting a poor outcome within the first day of a CA.¹⁴ When performing a clinical neurological examination, it is also of importance to take pharmacological interventions (sedation and analgesia) into account.

Electroencephalogram

Background

Conventional EEGs are obtained from multiple predefined locations covering the scull using the "10-20 system". A baseline recording is performed. In addition, the comatose patient is stimulated by sound or light to assess for reactivity. The EEG is sensitive to interferences, including breathing, muscular activity, and electrical fields. The EEG interpretation and analysis of artefacts is complex and usually preformed by a clinical neurophysiologist or a dedicated neurologist.

EEG in experimental CA

When eliciting temporary cerebral ischemia in animals, the EEG turns isoelectric 10-15 seconds after the circulatory arrest.⁹⁹ Recovery is then seen as intermittent cortical activity or SB which grows into continuous activity.^{100, 101} A study evaluating cats after global ischemia, showed that a normal recovery was distinctly different from a pathological recovery. In the latter case the initial EEG pattern was of a sudden onset high-amplitude pattern interposed with flat segments that disappeared after 3-5 hours, while the normal recovery comprised a low-amplitude activity that progressed to continuous activity with increasing frequency content.¹⁰² Experimental studies in rats have further demonstrated that the delay from start of reperfusion to recurrence of cortical activity correlates to neuronal destruction of the hippocampal CA1 region.¹⁰¹

Application of EEG in human CA

The development of EEG changes in humans has been studied in the setting of elective CA, while testing implantable cardioverters (ICDs).¹⁰³ Immediately after the CA, the EEG showed ischemic changes, with a slowing of frequency, and then turned isoelectric after 10-15 seconds. In these short CAs (i.e., 15-25 seconds), the EEG recovered to normal activity within about 20 seconds of ROSC. Thus, the initial EEG-development following ischemia is similar in humans and animals.^{99, 103}

Since the 60s, EEG has been evaluated as a prognostic tool for neurological outcome following CA.¹⁰⁴ Unfortunately, several classification systems have been proposed.¹⁰⁴⁻¹⁰⁶ These are not interchangeable and some EEG-patterns do simply

not fit the systems.¹⁰⁷ The literature is also hampered by a lack of standardisation, e.g., regarding timing between the CA and EEG evaluation. These problems have affected the use of EEG in prognostication of neurological outcome following CA.

The diversity in the literature has resulted in recommendations that an isoelectric EEG or a SB within the first week after CA,¹³ and SB or generalised epileptiform discharges, predict poor outcome, but with insufficient prognostic accuracy.¹⁰⁸ A scepticism regarding EEG as a prognostic tool is therefore not surprising. Recently, the prognostic value of testing the EEG for reactivity (to physical stimulation or eye opening) has been forwarded.¹¹⁰ In a retrospective study, a test 1-3 days after CA or return to normothermia correlated well with subsequent return of consciousness. A lack of reactivity correlated with continuous coma.¹¹¹

There are studies exploring the value of repeated EEG, focusing on EEGevolution, after CA.^{59, 94} Absence of cortical activity did not imply a uniformly poor prognosis, since 30% (37/125) recovered consciousness.⁵⁹ Similar to findings in cats,¹⁰² the EEG recovered via a phase of intermittent cortical activity to a continuous activity.⁹⁴ The median time from CA to recovery of continuous activity was 9 hours in a group of patients with initial cortical activity and 17 hours in a group with no initial activity.

The neurological and neurophysiological pattern of recovery was stereotypic in the initial phases, comprising first incomplete or complete recovery of brainstem reflexes and then recovery of cortical activity on the EEG. However, the timing of these events in relation to the CA differed, presumably as a reflection of the degree of the ischemic insult. The recovery of the EEG and of clinical signs could be halted at different stages in patients remaining in coma.⁹⁴ After return of consciousness, the change in EEG-findings and the clinical recovery occurred independently.⁹⁵

Continuous aEEG

Theory

To ensure simple long-term monitoring, a cerebral function monitor (CFM) was introduced in the 60s by Maynard *et al.*¹¹² It is based on amplitude differences of a few-lead EEG compressed over time, where longer time periods can be monitored and trends recognised. Technological development has made it possible to display a few-lead EEG registration and a compressed aEEG simultaneously (**Figure 1**).



Figure 1. The original EEG shown on the bottom of the screen corresponds to the vertical line (grey) on the aEEG at the top of the screen. Low-amplitude regions of the original EEG produce the lower limit of the aEEG (red), while the upper limit is produced by high-amplitude regions (black).

To reduce interference, an asymmetrical filter with profound filtration of high (>15/20 Hz) and low (<2 Hz) frequencies is incorporated. To increase sensitivity to detect ESE, the 10-15 Hz range is amplified. The amplitude-integration scale is linear from 0-10 μ V and semi-logarithmic from 10-100 μ V, to optimise visual interpretation. In neonates, usually one channel covering both hemispheres is used. In adults, we choose to use a two-channel set up with one lead covering the left and one the right hemisphere. A combination of the original EEG and the aEEG is used in the analysis. The display of the original EEG is important to identify artefacts. Interpretation by pattern recognition makes the aEEG-monitor a potential bedside tool in the ICU-setting (**Figure 2A-D**).



Figure 2A. Flat pattern. Amplitude integrated bands with baseline <5 (to 10) μ V.



Figure 2B. Suppression-burst pattern. Amplitude integrated pattern from <5 to >50 μ V. Distinct bursts inter-positioned with flat EEG in the original EEG.



Figure 2C. Electrographic *status epilepticus*. "Saw-tooth" pattern in the right half of the amplitude integrated display. Bilateral spike-waves in EEG tracing.



Figure 2D. Continuous pattern. Amplitude integrated bands with baseline >5 μ V. Symmetrical low amplitude continuous waveform in the original EEG.

Use in the neonatal setting

Amplitude integrated EEG has been used extensively in the neonatal setting. It was first employed to monitor seizures and has since been evaluated for early prediction of outcome in asphyxia.^{18, 112} A study by Toet *et al.* reported strong predictive values when relating the aEEG background pattern within six hours of birth to outcome. A flat trace, SB, or continuous extremely low voltage (about $5\mu V$) at six hours after birth had a positive predictive value of 86% and a negative predictive value of 91% for a poor outcome.¹⁸ Early aEEG-patterns have since been used to include or stratify asphyctic neonates regarding insult severity in clinical trials focusing on TH.^{88,89}

Use in cardiac arrest

aEEG has not been employed in adult global ischemia except during the initial evaluation.¹¹³ Instead, by tradition, conventional full-scale recordings have been used for prognostication in this group. However, there are similarities between the insult in neonates exposed to asphyxia and adults at CA, i.e., a transient global ischemic insult followed by reperfusion. This similarity, and the extensive local experience in using aEEG in the neonatal setting, formed the basis for **studies I** and **IV** of the present thesis. The literature in this area is sparse, but recently a study on long term EEG-monitoring (not aEEG) in hypothermia treated paediatric patients with CA was published.¹¹⁴



Figure 4. Example of a specific artefact pattern in aEEG-monitoring. A malfunctioning reference electrode was replaced, and the ongoing electrographic *status epilepticus* was detected.

Pitfalls

The aEEG-monitoring filters and amplifies minute electrical currents generated by the brain of the patient in an ICU environment with a high potential for electrical interference giving rise to artefacts. Common sources of error are loose electrodes or poor electrode connection, muscular artefacts from shivering, or movement artefacts in a patient regaining consciousness. In the case of malfunctioning electrodes the problem can usually be identified using the impedance check integrated in the monitor or by looking at the original EEG tracings (**Figure 4**).

BIS and entropy monitors

In search for tools that can predict neurological outcome following CA and TH, devices originally developed for assessment of depth of anaesthesia have been employed, i.e., "BIS" and "entropy" monitors. These monitors merely present an EEG as a score or as percentage of time spent in "electrical silence" (i.e., the suppression ratio or burst-suppression ratio). Quantitative "entropy" monitoring within 24 hours after CA has shown good correlation to outcome in a small study.¹¹⁵ BIS-recordings in muscularly relaxed patients early during TH also showed good correlation to outcome.¹¹⁶ While showing some promise, BIS monitors are critically hampered by the fact that the original EEG is not displayed, making any artefact analysis difficult. Furthermore, the identification of ESE is uncertain.¹¹⁷

SSEP

SSEP has been evaluated in CA arrest patients mainly prior to the introduction of TH. (At SSEP-measurements, forearm nerves are stimulated electrically and the impulse is recorded over *plexus brachialis* and the cerebral cortex.) A bilateral lack of the cortical N20 peak 24-72 hours after CA is a robust predictor of poor outcome (i.e., death or vegetative state).^{14, 118} The prevalence of bilateral lack of the N20 peak is 37-48% in patients remaining unconscious until death, and a preserved peak cannot be used to predict a good outcome.¹² The later latency peak, i.e., N70, has been evaluated in order to increase sensitivity,¹¹⁹ but instead this increases the false positive test rate.¹² During TH (24-28 hours after CA) SSEP showed a 100% specificity for continued coma, but the number of examined patients was still small.^{120, 121}

Biochemical markers

The ideal marker

A biochemical marker of brain injury should have certain properties.¹²² It should originate specifically in the brain and be released from neurons after irreversible cerebral damage with levels that correspond to the amount of damage. The sample material should be easy to access, e.g., blood, serum, or plasma, and the assay should be immediately available, reliable, and not sensitive to interactions caused by, e.g., haemolysis and hyperbilirubinemia. Finally, the marker should show clinical significance. Collecting CSF fluid by single or repeated lumbar taps has been used to gain access to material aiming at mirroring intracranial events.¹²³ However, CSF collection is potentially dangerous, especially in situations with heavy anticoagulation and in unconscious patients.

S-100B

Serum S-100 is a calcium-binding protein discovered in 1965.¹²⁴ It consists of two subunits (α and β) and is present in many isoforms. Several commercial tests for the β -form (S-100B) are available. S-100B in serum or CSF is often used as a marker of astroglial damage. The $t_{1/2}$ is about 30 min¹²⁵ and dependant on renal elimination.¹²⁶ Serum levels of S-100B have been examined in relation to outcome in several studies involving CA patients, indicating high levels in patients with poor outcome (*c.f.* good outcome). These studies have given rise to cut-off values for predicting poor outcome in non-TH^{17, 127} and TH patients.¹²⁸ However, S-100B is not specific for astrocyte damage. In the brain, the β -form is found not only in astrocytes, but also in other cells.¹²⁹ Furthermore, there is an extracerebral pool of S-100B. Most important of these sources in CA is probably the effect of release from fractures and, in the case of operated patients, fat tissue.^{130, 131} All together, this reduces the specificity of S-100B as a marker for cerebral damage. The analysis method of S-100B is also of importance if trying to establish cut-off values for a poor outcome in the CA situation.¹³²

NSE

NSE is an intracellular glycolytic calcium-binding protein with a $t_{1/2}$ of 30 hours.¹³³ It is present in neurons and other cells of neuroectodermal origin. NSE has been evaluated in CA. Cut-off levels predicting poor outcome with 100% specificity ranges between 25 and 33 µg/l in non-TH and TH patients,^{14, 16, 134} but considerably higher cut-off values have been suggested.^{135, 136} Compared to S-100B, NSE has a different time-profile in CA with brain injury.^{17, 137} Following CA and ROSC, the serum concentration usually rises after 24 hours to a high, sustained level at 48-72 hours. A rise in NSE between 24 and 48 hours has been associated to a poor outcome,¹³⁴ and a decrease of NSE between 24 and 48 hours

to a good outcome.¹²⁸ NSE is not neuron-specific, since it is also present in erythrocytes and platelets.¹³⁹ This is a source of error in the clinical setting, where even a subclinical haemolysis may affect the analysis.^{133, 140} There are several commercial analyses available, but they are not interchangeable.¹⁴¹

Other potential markers of cerebral damage

There are other potential markers for cerebral damage such as neurofilament, e-selectin, and glial fibrillary acidic protein (GFAP). These markers are so far, with few exceptions,¹⁴² unexplored in TH following CA.

Prognostication during therapeutic hypothermia

The addition of TH in post CA protocols complicates the evaluation since the use of sedation, analgesia, and intermittent neuromuscular blockade obscures neurological signs. Furthermore, the metabolism of propofol and midazolam is affected by the temperature reduction and their half-lives are prolonged.¹⁴³ Taken together, this has increased the interest in prognostication methods less sensitive to sedative medication than a clinical neurological examination. New objective prognostic markers are warranted and such tools may include neurophysiological methods such as aEEG and biochemical markers including S-100B and NSE. These potential tools are the focus of the present **studies I**, **II**, and **IV**.

Aims of the study

- i. To investigate the feasibility of aEEG and to study the relationship between aEEG and outcome after cardiac arrest and induced hypothermia (I).
- ii. To evaluate the development of EEG-patterns in relation to outcome in a larger cohort of cardiac arrest patients (IV).
- iii. To examine time profiles of NSE and S-100B in serum, and relating these to outcome in patients treated with hypothermia after cardiac arrest (II).
- iv. To evaluate the long term neurological outcome and recovery in hypothermia treated cardiac arrest patients (III).
- v. To summarise clinical, neurophysiological, biochemical, and radiological findings in patients remaining in coma after hypothermia treatment (V).

Materials and methods

The coma project

Background

When TH was introduced in clinical praxis in southern Sweden (Skåne) during 2002-2003, a decision was made to evaluate all treated patients. Furthermore, a thorough discussion focusing on inclusion criteria was undertaken. The published RCTs at the time both focused on unconscious adult OHCA patients of presumed cardiac origin with VT/VF as the presenting rhythm.^{9, 10} Extrapolating the findings of these studies to IHCA and to asystole/pulseless electric activity (A/PEA) was considered reasonable since the insult to the brain ought to be similar regardless of location of CA or presenting rhythm. Thus, IHCA and A/PEA patients were included into the programme. A discussion on whether to randomise the A/PEA patients to TH or not resulted a decision not to randomise these patients. The major reason for this was that a RCT involving a group of patients with expected mortality of 80-90% would demand a number of patients not available to us. However, we decided on a thorough follow up thus the "coma project" was born. Already from the beginning, the project was truly multidisciplinary, involving cardiologists, radiologists, neurologists, neurophysiologists, a neuropathologist, and intensive-care physicians.

Introduction of hypothermia treatment

Participating in the project were the General ICUs at Lund University Hospital, Malmö University Hospital, and Helsingborg General Hospital, as well as the Cardiothoracic ICU at Lund University Hospital, covering every adult patient with ROSC after CA and with sustained unconsciousness in a catchment area of approximately 800,000 inhabitants. Major effort was put into logistics within the Departments of Emergency Medicine, Cardiology, and Radiology in order to facilitate a smooth process from the emergency room to the ICU. This process included steps to get a fast cooling initiated in the emergency area or in the angiography laboratory, pre-prepared kits for blood sampling and TH induction, and major information "campaigns" to staff at all levels. A decision was made to treat the unconscious CA patients in the same way as any cardiac patient regarding emergency angiography and PCI.

Protocol

After resuscitation, ROSC, and initial stabilisation including endotracheal intubation, the following recommendations regarding TH was made:

- i. TH was *strongly recommended* in unconscious patients (GCS \leq 7 or RLS \geq 4), with a witnessed VT/VF, where the time from CA to ROSC was <30 minutes and TH could be initiated within 120 minutes of the CA.
- ii. TH was *recommended* to VT/VF patients with >30 minutes to ROSC and initiation of TH <4 hours of CA or unwitnessed VT/VF with <30 minutes to ROSC.
- iii. In cases of A/PEA, TH *could be considered* in patients with a witnessed CA and <30 minutes to ROSC. (TH in A/PEA patients with unwitnessed arrests was *not recommended*.)

Thus, in clinical praxis, TH was initiated on wide criteria compared to precedent studies.^{9, 10} Sedation was commenced by midazolam (approx. 0.05 mg/kg) and fentanyl (1-2 μ g/kg). Patients were relaxed using rocuronium (approx. 0.5 mg/kg), to reduce shivering and facilitate cooling. Cooling was initiated using 20-30 ml/kg cold saline i.v. (in praxis 2 l) given in about ten minutes,¹⁴⁴ and supported by cold fluid boluses, ice packs, or alcohol wiping at the discretion of the attending anaesthetist until maintenance cooling systems were instituted at the ICU. If angiography was indicated cooling was usually performed *en route* to the ICU.

In the ICU, TH was maintained by external (CritiCool, TREM, Israel or Arctic Sun, Medivance, CO) or intravenous (Icy Cath, Alsius, CA) systems. The patients were sedated using propofol (2-4 mg/kg/h) and fentanyl (1-2 μ g/kg/h). In cases of unstable circulation, with inadequate tolerance for propofol, midazolam (1-3 mg/h) was used for sedation. A MAP of >65 mmHg was aimed at, and inotropic, vaso-constrictor, and IABP support was used when necessary. Patients were normoventilated. Blood glucose levels were kept at 5-8 mM. Low-dose enteral nutrition was started. Potassium was kept at high normal levels (4-5 mM).

Patients were kept at $33\pm1^{\circ}$ C for 24 hours. Rewarming was active and controlled to 0.5° C/h. Sedation was minimised at normothermia (>36.0°C). In patients reaching normothermia during night, sedation was not always reduced until the following morning. After normothermia, sedation was kept to a minimum to facilitate mechanical ventilation until extubation. At normothermia, a three days observation period was allowed. In patients not regaining consciousness, an MRI and an SSEP was carried three days after normothermia. Thereafter, a neurological examination was performed. In patients with a GCS-motor status of 1-2 or bilateral lack of N20 peak in SSEP at this time-point withdrawal of intensive care was recommended. In cases of uncertainty the observation period was prolonged.

Patients were assessed using the cerebral performance categories scale (CPC scale).¹⁴⁵ The assessments were performed when the patient left leaving the ICU, when he/she left the hospital, and at six months follow up.

The CPC scale is a five-graded scale:

- 1. Good cerebral performance.
- 2. Moderate cerebral disability, independent.
- 3. Severe cerebral disability, conscious but dependent.
- 4. Coma.
- 5. Death.

A neurologist and an occupational therapist performed an evaluation covering neurological status, cognitive, processing, frontal lobe, and memory functions six months after the CA. In patients living abroad or in distant places, the follow up was made by phone, interviewing the patient, relatives, and his/her local doctor.

Continuous aEEG

Study I and **IV** comprised patients at the general ICU at Lund University Hospital. The aEEG registrations were carried out at the ICU. When a CA patient had been stabilized, a two-channel aEEG recorder was connected to the patient (Nervus Monitor, Viasys Healthcare, WI). Four subcutaneous needle electrodes were placed at central and parietal positions on each side of the scull (C3-P3 and C4-P4 according to the "10-20 system"), in addition to a combined frontal electrode functioning as reference and ground.

In **study I**, the initial four-hour aEEG-tracing (based on neonatal studies),¹⁸ and the corresponding tracing at resumption of normothermia (to reduce possible interference of reduced temperature), were analysed. The attending physicians were blinded to the Nervus-monitor and the neurophysiologist to clinical features. In **study IV**, the aEEG was analysed at the same points in time, but emphasis was also put on the development of the EEG/aEEG tracings during and after TH. For example, all measurements were screened for ESE. Major patterns, i.e., flat, SB, ESE, and continuous EEG, were defined using amplitude and frequency by the combination of EEG/aEEG prior to analysis. The neurophysiologists were blinded to the clinical features of the patients, but due to the ease of pattern recognition, blinding of the attending physicians was impossible after the first 34 patients (who took part in **study I**). The aEEG patterns were related to recovery of consciousness.

Biochemical markers

Study II comprised patients from all units participating in the coma project. Serum samples for NSE and S-100B analysis were drawn from an arterial line at 2 ± 1 , 24 ± 4 , 48 ± 4 , and 72 ± 4 hours following CA. In addition, a sample was obtained in the emergency room to secure an early sample. These samples were usually venous since an arterial line had not yet been established.

NSE-samples were centrifuged and refrigerated if analyzed within 24 hours. Otherwise they were frozen (-20°C) and analyzed within a week. The LIAISON NSE, an immunoluminometric method, was used for the analysis (DiaSorin, Sundbyberg, Sweden). The detection limit of the assay is 0.04 μ g/l and the upper reference level for human serum 12.5 μ g/l. Samples with visible haemolysis were discarded.

The S-100B samples were centrifuged and the analysis was done within 24 hours. Samples were kept at room temperature if time from sampling to analysis was less than eight hours. The LIAISON S-100B, an immunoluminometric method, was employed for analysis of S-100B (DiaSorin). The detection limit is <0.02 µg/l and the upper reference level for human serum <0.15 µg/l. The S-100B study was prematurely stopped due to a change in S-100B analysis method at the Department of Clinical Chemistry. The material was dichotomised into a good and a poor outcome group, based on best CPC during the six months follow up period. A best CPC 1-2 was regarded as a good outcome and a best CPC 3-4 as a poor.

Follow up

Study III comprised patients from all units participating in the coma projects. Neurologists and occupational therapists conducted a follow up at six months after the CA. Apart from a neurological examination and classification into the CPCscale, this follow-up included tests and self-assessments focusing on:

- i. Memory capacity.
- ii. Motor and processing skills.
- iii. Frontal lobe functions.
- iv. Global cognitive difficulties.
- v. Anxiety, sleep, and depression scales.

After the conclusion of **study III**, TH patients from Lund continued with the same follow up program until 2008. In patients living abroad or in remote areas, the follow up was predominantly performed over the phone by contact with the patient, his/her relatives, and his/her local physician. This follow up was performed by an intensive care physician.

Patients remaining in coma

Study V comprised consecutive patients at the general ICU at Lund University Hospital. The study was aiming at a descriptive characterisation of patients remaining in coma when the neurological examination three days after normothermia was performed, using results from NSE, aEEG, SSEP, MRI, and, if available, a *post mortem* morphologic evaluation.

The result of the neurological examination was extracted from the patient files. Patients still unconscious three days after the return to normothermia were included. From the neurological examination, the pupillary and corneal reflexes were noted and regarded as absent if bilaterally absent (otherwise present). The GCS motor response was noted.

Based on the findings in **study II**, serum NSE-levels at 48 hours were selected as the comparative biochemical marker. The dominating aEEG-pattern after normothermia was assessed by a neurophysiologist (blinded to clinical data) and was reported as flat, SB, ESE emerging from a SB or a continuous background, and continuous. The result of the N20 median nerve SSEP was reported as bilaterally absent or not bilaterally absent. A neurophysiologist blinded to patient characteristics made the evaluation.

A single neuroradiologist, blinded to patient evaluation and outcome, assessed the diffusion-weighted MRI-scans. The scans were divided into 3 groups: (i) normal, (ii) infarction volume <20 ml, and (iii) infarction volume >20 ml. The neuro-pathological examination was made without knowledge of the clinical, radiological, or neurophysiological findings. The results of aEEG, SSEP, MRI, neurological examination, and neuropathological findings were related to the NSE-levels 48 hours after the CA.

Results and discussion

Continuous aEEG

In **study I**, 34 patients with CA were included from Jan 2004 to Feb 2005. All tracings were interpretable. All 20 patients with continuous aEEG at normothermia eventually regained consciousness, while none of the 14 patients with SB, flat, or electrographic *status epilepticus* (ESE) regained consciousness.

Seven out of thirty-four patients (21%) developed an ESE prior to normothermia. The pattern at start of registration was predominantly flat (in 24/34 individuals), and could develop into any other pattern. In contrast to results in neonates,¹⁸ an initial flat pattern seemed to be of little predictive value in adults.

The findings in **study I** strongly suggested, for the first time, that aEEG had a unique prognostic potential for neurological outcome at TH following CA. This prompted further investigation, i.e., **study IV**, which differed from **study I** regarding number of patients and how the aEEG was analysed.

In **study IV**, 95 patients receiving TH were included. The aEEG was started at a median eight hours after the CA. In addition to an analysis during four hours at the start of monitoring and during four hours of normothermia, the EEG was analysed for EEG/aEEG progression between these observation points and thereafter.

A continuous initial aEEG pattern was strongly associated with recovery of consciousness (PPV 91%, CI 75-98%). The prevalence of an early flat aEEG was high (47/95) and not related to outcome. During TH and ongoing sedation 28 of the 47 patients with an initial flat aEEG pattern regained a continuous aEEG.

The sequence of recovery was in agreement with previous reports using intermittent EEG, i.e., the initial flat pattern developed into a continuous pattern via a period of intermittent cortical activity.^{59, 94, 102}

Patients with an initial SB, or developing a SB-pattern from a flat pattern, usually turned into an ESE. The start of SB was usually abrupt, similar to findings in ischemically compromised cats.¹⁰² No patient with a SB-pattern at any time regained consciousness, which is in accordance with the literature.^{14, 118} SB was an

unstable pattern since no patient, who was still monitored, stayed in SB 24 hours after NT, which is in agreement with findings of Thömke *et al.*¹⁴⁶

The most stable pattern during TH and after normothermia was a developed continuous pattern. At normothermia, this had a high PPV (87%, CI 76-94%) for a later recovery of consciousness. Thus, for the first time, a strong positive predictor for recovery of consciousness was identified.

The entire lengths of all recordings were analysed for ESE. Twenty-eight percent (26/95) developed an ESE during or after TH, an incidence similar to what has been described previously.¹¹⁰ The ESE patients could be arranged into two groups, i.e., those developing ESE from a SB background (16/26) and form a continuous background (10/26) (**Figure 5**). The former group fulfilled ESE-criteria earlier, i.e., a median 5.5 hours prior to normothermia vs. 3.5 hours after normothermia (p=0.01).

At the time of normothermia, the incidence of ESE increased (**Figure 5**), similar to findings in paediatric CA patients treated with hypothermia.¹¹⁴ The reason for this is unknown, but explanations may be a reduction of the membrane stabilising effect of hypothermia,¹⁴⁷ a concurrent reduction in sedative medication, an effect of the natural course of the global ischemic injury (in parallel to what is seen in asphyctic neonates),⁸⁷ or a combination of these factors. Two patients with ESE regained consciousness (CPC 2 and 3 at 6 months, respectively), both of them developed ESE from a continuous background. This is not previously described and if verified it may have implications for selection or stratification of patients in future treatment studies.

In the present study, we did not test for EEG reactivity since the focus was on the early time period following CA during the hypothermia phase when ongoing sedation would reduce the reliability of reactivity tests. The aEEG-monitoring provided a continuous monitoring for a development of ESE. In turn, this could result in early institution of anti-epileptic medication and for continuous monitoring of such measures.

Current treatment recommendations for refractory *status epilepticus* includes treatment with thiopenthal, midazolam, or propofol for 24 hours aiming at SB.¹⁴⁸ This demands a continuous monitoring and dose adjustment,¹⁴⁹ and aEEG may be a tool by which this can be achieved. No trials focusing on treatment of refractory *status epilepticus* secondary to global ischemia have been published.



Figure 5. Start of electrographic *status epilepticus* (ESE) in relation to time for normothermia (NT). "ESE from SB" denotes ESE starting from a SB background, and "ESE from con" denotes ESE starting from a continuous background. Note the high incidence of ESE at the time of NT. Arrows indicate the only surviving patients.

In conclusion, the present aEEG monitoring originally suggest that aEEG is a valid prognostic tool for early prediction of neurological outcome during TH following CA. In CA patients receiving TH, aEEG-monitoring may facilitate decisions regarding patient care and result in improved information to relatives.

Biochemical markers

For the NSE study, 107 patients were recruited from the General ICU at Lund University Hospital from 2003 to 2007. For the S-100B study, 116 patients were recruited from all participating units from introduction of TH in the respective unit to Jan 2005. The patients were enrolled consecutively.

The main findings in study II were:

- i. A statistically significant change of NSE-levels over time in the poor outcome group.
- ii. Statistically significant differences in NSE between the good and the poor outcome groups at 24, 48 and 72 hours after CA.

A rise in NSE >2 μ g/l between 24 and 48 hours after CA was strongly associated with poor outcome (OR=9.8, CI 3.5-27.7). Cut-off values with a high specificity were identified: at 48 hours an NSE >28 μ g/l had a 100% specificity and a 67% sensitivity for a poor outcome. The cut-off values at both 24 and 72 hours were higher, and consequently sensitivity was impaired. For S-100B, there were statistically significant differences at all sampling times between the good and the poor outcome groups. The best cut-off value for S-100B was 0.51 μ g/l at 24 hours following CA (specificity 96%, sensitivity 62%).



Figure 6. Development of NSE over time following cardiac arrest. Statistically significant differences in NSE between groups with good and poor outcome were observed at 24 through 72 hours. Medians, interquartile ranges, and ranges excluding outliers are indicated. Outliers are shown separately. To put focus on lower NSE levels, NSE values >200 μ g/l were omitted (i.e., three values from the poor outcome groups at 48 and 72 hours).

There are weaknesses with both NSE and S-100B as prognostic markers for neurological outcome at TH following CA. Regarding S-100B, the protein is not astrocyte specific. Thus, it may spill over into the circulation from fractures¹³⁰ and mediastinal fat,¹³¹ relevant to cardiopulmonary resuscitation. Taken together, in CA following resuscitation, we do not know accurately whether the measured S-100B is derived from astroglial cells or from extracerebral sources.

It is our view that increased serum levels of NSE at 48 hours, and increasing levels over time, can be used as an adjunct in prognostication of neurological outcome at TH following CA. However, NSE-levels must be viewed with caution in patients with extracorporeal circulation or IABP due to the risk of artefacts associated with haemolysis. Reflecting this, our opinion is that the use of single serum samples should be discouraged. The difference between the commercially available NSEanalyses, and possible inter-laboratory variations, reduce the generalization of our results.¹⁴¹ This can be exemplified with the findings of Reisinger et al. (using a different analysis than ours), who reported a 100% specificity and 63% sensitivity for a peak NSE-concentration of 80 µg/l during the first four days following CA in unconscious non-TH patients.¹³⁵ The outcome measure was patients regaining consciousness, i.e., CPC 3 or better. Using our data with a peak cut-off of 80 μ g/l, would result in a sensitivity of 30%. Also, the median and IQR were substantially lower in our poor outcome group compared with Resinger's results (39 µg/l, 22-94 $\mu g/l$ vs. 132 $\mu g/l$, 49-215 $\mu g/l$). Using a best CPC 3 in our data as a good outcome would not have changed these results.

Follow up

During the inclusion time 88 patients were treated with hypothermia in the three centres. Six months after the CA, 48 patients (55%) were alive. Forty-three patients were included in the neurological follow up, 36 (84%) were in CPC 1, 6 (14%) in CPC 2, and 1 (2%) in CPC 3. All were living at home, albeit one with extensive help. Nine patients had undergone rehabilitation for neurological symptoms prior to follow up. In the majority of cases (36/43), the motor neurological evaluation was unremarkable. A majority had distinguishable difficulties in higher functions, often affecting long-term memory (approximately 33%), frontal lobe function (37%), and moderately (21%) to severely (26%) affected processing skills. In contrast, sleep and depression indices were comparable with findings in elderly populations.

A minority of the patients had returned to work (8/19), which is in agreement with findings in another Swedish material.¹⁵⁰ In contrast to that study, no patient lived

in a nursing home, which may have been due to the amount of support from relatives or social services.

In long term follow up after CA, the majority of patients do well according to the CPC scale both prior to and after the introduction of TH.^{2, 151} TH does not seem to increase the cognitive dysfunction compared to non-TH CA patients.¹⁵² Cognitive dysfunction after CA is common, but in most cases it can be managed with instruction in compensatory strategies.

Bedside screening during the first month after CA has been shown to identify patients at risk for long term neuropsychological dysfunction.¹⁵³ Based on the findings in **study III**, we have individualised the follow-up program. To detect patients at risk of advanced long-term cognitive dysfunction, and to institute rehabilitation, we have moved the screening from six to two months after the CA. This includes a mini-mental test to screen for memory disturbances and SF-36, an instrument measuring self-reported physical and psychological health validated for a Swedish population.¹⁵⁴ Patients with abnormal results on these test, and patients who complain of cognitive disturbances, are referred to an occupational therapist and a neurologist in order to institute rehabilitation and long-term follow-up.

Patients remaining in coma

In **study V**, 111 were patients included. Three days after normothermia, 58 patients had regained consciousness, 19 were dead, and 34 remained unconscious. At six months follow up, 54/111 (49%) had made a good recovery (CPC 1-2), three had severe sequels (CPC 3), while the remaining 54 patients were dead. Among the 34 patients in coma three days after normothermia 28 patients remained unconscious, and six regained consciousness.

NSE, the only continuous variable, was used to rank the unconscious patients. Patients with high NSE-levels (i.e., >33 µg/l) also had other evidence of ischemic lesions in multiple modalities, reflected by strong correlations between NSE and MRI, SSEP, and neuropathology findings, respectively. In patients with intermediary NSE-levels, i.e., 27-33 µg/l, the multimodal evidence of cerebral ischemia was less common, and in the low NSE-region (i.e., <27 µg/l), the patients neither had lack of pupillary or corneal reflexes nor pronounced ischemia on MRI or bilateral lack of SSEP-findings, suggestive of extensive cerebral ischemia.

In the patients with NSE <27 μ g/l, 8/13 patients were deeply comatose (at best extension pattern on pain stimulation) and all of these had an ESE: one regained

consciousness. The remaining 5/13 unconscious patients had a continuous EEG as the dominating pattern and were less deeply comatose: all eventually regained consciousness.

Our data support the findings of Al Thenayan *et al.*, suggesting that a TH-patient with extension pattern as long as five days after the CA may regain consciousness.¹⁰⁹ This adds uncertainty regarding the clinical neurological examination and favours a multimodal approach for prognostication after TH. We also, as expected,¹⁴ found a low prevalence of lack of pupillary or corneal reflexes.

The major findings in this study are the concordance of the diagnostic modalities used among patients with high NSE levels, the importance of EEG evidence of ESE in handling unconscious patients, and the fact that the patients may have a post-CA ESE with normal findings on SSEP, NSE, and MRI.

Ethical aspects and withdrawal of intensive care

When evaluating patients with an expected combined mortality and vegetative state rate of approximately 80%,^{60, 61} the decision process in limiting support in the ICU is important. The praxis of end-of-life decisions differ considerably between countries with different geographical and religious background.¹⁵⁵ Also, a change over time in the praxis of withdrawal and withholding therapy is evident.¹⁵⁶ This, in combination with quality of care, results in long-term vegetative rates as low as $0.4\%^2$ to as high as 18%.¹³⁶ It is now difficult to find recent studies on CA outcomes without limitations of care. However, one such study involving 43 patients with IHCA receiving TH reported a 69% mortality and 9% persistent vegetative state at a six months follow up.¹⁵⁷

In the present study, a decision to withdraw intensive care due to predicted poor neurological outcome was made at the earliest three days after return to normothermia. The timing was based on precedent (up until 2003) results from major studies indicating the prognosis after CA.^{13, 96-98} In addition, we decided to allow time for the sedation used in TH to wear off and other (unknown) potential effects of TH to manifest themselves. Therefore, the time of TH was added to the three days observation time, resulting in a decision point in time 4.5-5 days after the CA (and this is now our praxis). Finally, since bilateral lack of N20 SSEP within the first week after CA was considered a strong predictor for a poor neurological outcome,¹³ an SSEP recording was added to the clinical neurological examination (three days after normothermia).

Taken together, in unsedated patients, deeply comatose (at best extension pattern on pain stimulation) three days after normothermia, or presenting bilateral lack of N20 SSEP, withdrawal of intensive care was recommended on the grounds of neurological futility. In cases of uncertainty, the observation period was prolonged. Withdrawal of intensive care included tapering off and stopping ongoing vasopressor or inotropic support and extubation with a continued free airway (nasopharyngeal or oropharyngeal) if necessary. In patients leaving the ICU after withdrawal of intensive care, a decision not to return to the ICU was included. A decision to withdraw intensive care always implied continued palliative care. This might include opioids, benzodiazepines and seizure medications. During the entire ICU stay, the most accurate information available was relayed to the patient's relatives.

A decision to give unlimited intensive care would have strengthened the present conclusions an avoided any risk for a "self-fulfilling prophecy", i.e., that premature withdrawal of intensive care leads to the death of the patient. However, in the Swedish health care system, unlimited intensive care for CA is not considered ethical neither in relation to the comatose patient (futility) nor to other patients who might be in need of intensive care. However, extending the observation period to 4.5-5 days after the CA before making a statement on prognosis, and extending the observation time in cases of uncertainty, seemed reasonable in the light of previous studies^{13, 96-98}.

Finally, in a clinical context, a number of CA patients are elderly with serious concurrent diseases. It may even become clear that they have had end-of-life discussions with their relatives. These factors may influence the decision to withdraw intensive care in the individual patient

Future directions

Biochemical markers

NSE

Further studies are warranted employing NSE as prognostic marker for neurological outcome in TH following CA. Importantly such studies should test a preset cut-off limit.

Soluble neurofilament

A neuronal marker for brain damage that is measurable in serum is soluble neurofilament. An evaluation of the potential for use in a CA situation is currently ongoing.

EEG

Further studies are warranted employing aEEG as prognostic marker for neurological outcome in TH following CA. Such studies should be prospective and use standardised neurophysiological definitions.

An interesting extension of the current work would be to examine whether or not the interval between CA and re-appearance of continuous aEEG activity is correlated to final outcome among patients regaining consciousness.

Finally, it would be interesting to prospectively study whether or not the reactivity pattern of the EEG (after accomplished TH) has any prognostic value in TH following CA.

Conclusions

- i. Long term aEEG monitoring was feasible in the ICU in patients receiving therapeutic hypothermia (TH) following cardiac arrest (CA).
- ii. A continuous aEEG-pattern either at start of registration or at normothermia strongly correlated to recovery of consciousness, while other patterns at normothermia strongly correlated to continued coma. Electrographic *status epilepticus* was common.
- iii. Of the biochemical markers, NSE was superior to S-100B. NSE levels over $28 \mu g/l$ at 48 hours after CA as well as an increase in NSE levels between 24 and 48 hours were strong predictors for a poor outcome.
- iv. Fifty-five percent of the patients survived CA, 98% of whom lived an independent life six months later. Cognitive dysfunctions were common, mainly affecting memory functions and processing skills.
- v. A majority of patients remaining in coma 4.5 days after CA had multiple clinical, neurophysiological, biochemical, or radiological findings indicating advanced ischemic brain injury, and none of these regained consciousness. The remainder were in coma without multiple findings of ischemic brain injury. These patients either had electrographic *status epilepticus* (12.5% regained consciousness) or a continuous EEG pattern (100% regained consciousness).



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Populärvetenskaplig sammanfattning

Personer som drabbas av hjärtstopp riskerar grava hjärnskador p.g.a. brist på blodflöde till hjärnan. Patienterna blir ofta långvarigt medvetslösa och vårdas på intensivvårdsavdelning i respirator. Risken för hjärnskada kan minskas med kylbehandling. Under kylbehandlingen, cirka 1.5 dygn, får patienten sederande och ev. muskelavslappnande medicin för att minska "huttring". Medicineringen påverkar möjligheten att göra en neurologisk värdering under kylbehandlingen och den närmsta tiden därefter. För att kunna ta ställning till fortsatt vård och ge bästa möjliga information till anhöriga krävs därför andra prognostiska metoder. Målet med avhandlingen var att undersöka kontinuerligt amplitudintegrerat EEG (aEEG) samt hjärnskademarkörerna neuronspecifikt enolase (NSE) och S-100B som tidiga markörer för hjärnskada hos kylbehandlade patienter.

Studierna omfattade kylbehandlade patienter i Lund, Malmö och Helsingborg under åren 2003-2008. Kontinuerligt amplitudintegrerat EEG (aEEG) kopplades upp då patienten anlände till intensivvårdavdelningen och kopplades ner då patienten återfick medvetandet eller, vid fortsatt medvetslöshet, efter 120 timmar. Serumprover för hjärnskademarkörerna NSE och S-100B togs vid upprepade provtagningstillfällen under de tre första dygnen efter hjärtstoppet. För de patienter som inte återfick medvetandet kompletterades ovanstående med neurologisk undersökning, magnetresonanstomografi av hjärnan (MR) och somatosensorisk evoked potential (SSEP) tre dygn efter kylbehandlingens avslutning. (Vid SSEP stimulerar men nerver i handleden elektriskt och mäter hur nervimpulserna fortleds till hjärnan.) Överlevande patienter följdes upp sex månader efter hjärtstoppet.

De viktigaste resultaten var:

i. att ett kontinuerligt aEEG-mönster vid kylbehandlingens avslutning talade starkt för att patienten skulle återfå medvetandet, medan andra aEEG-mönster talade starkt för fortsatt medvetslöshet.

- ii. att en status epilepticus-bild i aEEG sågs hos 28% av patienterna och att detta innebar en hög risk för fortsatt medvetslöshet: endast 7% av dessa patienter återfick medvetandet.
- iii. att NSE-värden över 28 μg/l 48 timmar efter hjärtstoppet, respektive en ökning mellan 24 och 48 timmar efter hjärtstoppet, talade starkt för dålig prognos.
- iv. att cirka 50% av de patienterna var i livet sex månader efter hjärtstoppet och att 97-98% levde ett självständigt liv. Trots detta kunde brister i framför allt minnesfunktioner konstateras. 2-3% hade kraftigt nedsatt funktionsnivå.
- v. att 34 av 111 patienter som var medvetslösa tre dygn efter avslutad kylbehandling hade tre typer av skademönster:
 - Multipla tecken på hjärnskada i form av mycket djup medvetslöshet, bortfall av SSEP, utbredda skador på MR och gravt patologiskt aEEG. Inga av dessa patienter återfick medvetandet.
 - Mycket djup medvetslöshet med status epilepticus bild i aEEG utan andra tecken på utbredd hjärnskada. Endast en av dessa patienter återfick medvetandet.
 - Ytlig medvetslöshet med kontinuerligt aEEG-mönster. Dessa patienter återfick alla medvetandet.

Sammanfattningsvis kan kontinuerligt aEEG och NSE ge tidig information om patienten som kylbehandlas efter hjärtstopp kommer att återfå medvetandet eller inte. Hos patienter som är långvarigt medvetslösa kan en kombination av undersökningar sannolikt underlätta värderingen av patientens prognos. De patienter som återfår medvetandet kan i de allra flesta fall återgå till ett självständigt liv.

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Paper I

Paper II

Paper III

Paper IV

Paper V