

PROTOKOL

**Time-differentiated treatment with mild therapeutic hypothermia, a multicenter
study**

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Abbreviations

ACE	Addenbrook's cognitive examination
BAEP	Brain auditory evoked potentials
BNP	Bain natriuretic peptide
CRF	Chart record form
CPC	Cerebral performance category score
EEG	Electroencephalogram
EKG	Electrocardiogram
EMG	Electromyogram
ERP	Event related potentials
FATE	Focus assessed transthoracic echo
GCP	Good clinical practice
GCS	Glasgow coma score
KT	Colloid osmotic pressure
KAG	Coronary angiography
MMSE	Minimal mental state examination
NIRS	Near infrared spectroscopy
NSE	Neuronspecific enolase
PAID	Paroxysmal autonomy instability with dystonia
ROSC	Return of spontaneous circulation
SSEP	Somato- sensory evoked potentials
VF	Ventricular fibrillation
VT	Ventricular tachycardia

Synopsis

In Denmark there are approximately 3,500 cardiac arrests annually. The mortality rate is high, less than 10% of the patients survive in the long term and many of them with neurological sequel. The reason for the high mortality rate is the lack of oxygen supply to the brain, and therefore the resulting brain damage. In 2002, two randomized studies, proved that mild therapeutic hypothermia, where the body temperature is cooled down to 33 ° C, improves cerebral outcome, measured by Cerebral Performance Category Score (CPC). However, it is unknown how long the optimal period of hypothermia is. In the two studies from 2002, patients were cooled for respectively 12 and 24 hours. In newborns with cerebral anoxic damage, cooling treatment for 72 hours is recommended. With that in mind, it is reasonable to assume that cooling beyond 24 hours in adults might improve cerebral outcome further.

The aim of this study is to compare 24 versus 48 hours of therapeutic hypothermia with a target temperature of 33 ° C. **The hypothesis** is that cooling for 48 hours results in a cerebral outcome, which is better than when cooling for 24 hours, measured by CPC. The study is a clinical, randomized multicenter study. Measuring of the primary endpoints CPC 6 months after the heart attack is performed blinded. Secondary outcome parameters are side effects following the treatment, GCS, and CPC at discharge, after 28 days and after 3 and 6 months.

Inclusion criteria's: Out of hospital cardiac arrest with suspected cardiac cause. Restored circulation after resuscitation (When CPR is not necessary for 20 minutes and there are clinical signs of circulation). Glasgow Coma Score (GCS) <8. Estimated time interval from collapse to acceptable circulation ≤ 60 min. Age ≥ 18 years. Age <80 years. For exclusion criteria's, see the protocol. Based on a 2-sided statistical power calculation, the study includes 350 patients, whereby it will be possible to detect a difference of 15% or greater between the groups, with a power of 0.8 and a significance level of 0.05.

The study is divided into a basic study, which encompass all study-centers and includes intervention with 24 or 48 hours of hypothermic treatment, standard cooling therapy and monitoring of the primary and secondary outcome parameters.

Moreover, several additional studies are described, which exclusively include expanded monitoring without further intervention, are described. Each individual study-center can voluntarily decide whether they wish to participate in this part of the study or not.

The study has been approved by the ethical committee and reported to the Data Supervision and clinicaltrials.gov.

Background

Epidemiology

In Denmark there are approximately 3,500 cardiac arrests annually. The mortality rate is high with only a 10 % survival rate in the long term (1), and many with neurological sequelae (2). One reason for the high morbidity and mortality is the brain damage that occurs due to the lack of oxygen supply to the brain.

Pathophysiology

Following circulatory failure, as seen with cardiac arrest, the brain's energy stores (glucose and ATP) will be consumed within 5 minutes (3). Hypoxia in the tissue and energy deficiency lead to loss of the electrochemical transmembrane gradient and hereby failure of synapse-transmission and generation of action potentials (4). The released glutamate and intracellular calcium accumulate, which lead to cell death (5). After restoring circulation, reperfusion and reoxygenation lead to further nerve cell damage (reperfusion injury). The cerebral microcirculation fails with primary hyperemia to follow, and later global and multi-focal hypoperfusion. This happens because of vasomotor paralysis (6). Reoxygenation also starts a chemical cascade with the production of reactive oxygen species that cause oxidative damage. Changes in the inflammatory response can cause activation of endothelial cells, infiltration of leucocytes, and further tissue damage (7).

Treatment

The treatment of brain damage has for many years largely been the same with no significant progress or changes. Throughout the 1980s and the 1990s animal studies showed, that treatment with mild hypothermia after cardiac arrest could improve the cerebral outcome.

Hypothermia reduces the metabolism of the brain including oxygen and ATP consumption (8,9). At the same time release of glutamate and dopamine is reduced (10). The oxidative stress is weakened and lipid peroxidation is reduced (11). Apoptosis is inhibited due to the reduction of both calcium-accumulation and glutamate-release. Hypothermia suppresses also the inflammatory response and reduces at the same time the early hyperemia and the later hypoperfusion (12,13).

In 2002 "The New England Journal of Medicine" published two human studies (14,15), which showed, that treatment with mild hypothermia for 12 - 24 hours of out of hospital cardiac arrest victims caused by ventricular tachycardia (VT) or ventricular fibrillation (VF), improved cerebral outcome. Furthermore one of the two studies showed a significantly improvement of the mortality. Both studies were randomized and partially blinded. The two studies were respectively an European multicenter study and an Australian study. Based on these facts, it is demonstrated, that mild therapeutic hypothermia after cardiac arrest improves patient's outcome. Cerebral outcome was in the two studies measured by "Cerebral Performance Category Score" (CPC) (16) and mortality. These are relatively primitive, but easily manageable clinical parameters.

The results led to, that ILCOR (International Liaison Committee on Resuscitation) recommended hypothermic treatment for adults with out of hospital cardiac arrest of presumed cardiac genesis and with ventricular fibrillation (VF) or ventricular tachycardia (VT) as the causative arrhythmia. (17). In 2013 a multicenter study examined two different temperatures regiments. 950 out of hospital

cardiac arrest patients were randomized to 24 hours of hypothermia at 33 or 36 degrees Celsius respectively. The study showed no difference between the two arms in CPC or adverse events 6 month after the collapse (60). Following publication of the trial ILCOR stated that “pending formal consensus on the optimal temperature, we suggest that clinicians provide post resuscitation care based on the current treatment recommendations (33°C). We accept that some clinicians may make a local decision to use a target temperature of 36°C pending this further guidance.”

Side effects

With respect to adverse events, the two randomized studies (14,15) found an increased frequency of pneumonia and sepsis in the group treated with hypothermia compared to those treated conventionally. The difference was although not statistically significant. In addition, it is known that hypothermia affects many functions of the organs in the body, as well as the physiological system generally, including stress response and coagulation.

Unresolved treatment regimens

An important question is how long hypothermic therapy should be maintained. One of the studies cooled the patients for 12 hours, the other one for 24 hours. However concerning neonates, there is evidence-based proof of cooling patients with anoxic brain injury for 72 hours (18). Therefore, it is unclear whether the optimal duration of hypothermic therapy concerning adults is 12, 24, 48 hours, or a another time interval. Despite these facts, it is reasonable to assume, that an extension of hypothermic treatment beyond 24 hours, which has become the standard treatment in Denmark, might provide further improvement of the cerebral outcome. Additionally it must be assumed that the risk of side effects in relation to hypothermic treatment are increased with prolonged cooling. Whether the cerebral outcome improves correspondingly or even more is unknown.

The overall purpose of the study is to investigate this problem.

There are other unresolved questions related to hypothermic treatment (19,20). It is obvious that patients cannot wait for days before the treatment is initiated. Intuitively treatment should be started as soon as possible, but there is no evidence of this. There is a single retrospective study in which the cerebral outcome is negatively correlated with the time starting from the cardiac arrest and until the target temperature for hypothermic treatment is reached (21). Conversely, a smaller Danish cohort study and a larger observational multicenter study, could not demonstrate differences in cerebral outcome as compared to elapsed time from collapse to achievement of the target temperature (22, 23). The "Time window" for punctual hypothermic treatment has therefore not yet been clearly defined. It is equally unclear which target temperature is best. In the two afore mentioned randomized trials (14,15) the target temperature was 32 -34 ° C, but whether a temperature of 35 or 31 ° C will provide better outcome is unknown.

It is shown, that respectively ROSC (Return of Spontaneous Circulation), BLS (Basic Life Support), age and initial rhythm, all affect both cerebral outcome and mortality after cardiac arrest (23,24).

Methods for the assessment of cerebral outcome

To comment on cerebral outcome it is important to have an extensive and detailed monitoring of the cerebral function following the cooling treatment. Monitoring of the cerebral function can be performed in a variety of ways.

-CPC and GCS

Cerebral Performance Category Score (CPC) (16) and Glasgow Coma Score (GCS) (25) are relatively simple, clinical parameters used in previous studies.

-Cognitive function

More specific and detailed information can be obtained by evaluation of the cognitive function. The basic cognitive function can be evaluated with a Minimal Mental State Examination (MMSE) combined with Addenbrook's Cognitive Examination (ACE). While an evaluation of an enhanced cognitive function can be done with a "test-battery" with a total "sum-score". These tests are standardized neuropsychological tests, which measure intelligence and cognitive domains. The tests are designed for covering a wide range of cognitive skills, and have previously been shown to be clinically sensitive. They are able to detect even subtle cognitive deficits in patients with a slightly cerebral affection, which is seen as for instance after a minor head trauma (26-36). With these tests, it will be possible to refine the more basic neurological GCS and CPC evaluation.

-SSEP (somatosensory evoked potentials)

The use of SSEP as a prognostic marker is based on a high degree of evidence. A class I study and 7 Class III studies showed, that a lack of bilateral cortical SSEP has a high predictive value, where the lack of SSEP is associated with poor outcome (persistent vegetative state or death) with a rate of 0 concerning false-positives (37). During hypothermic treatment the above statement still appears to be valid (38).

-Autonomic dysfunction (PAID)

Paroxysmal Autonomic Instability with Dystonia (PAID) typically occurs in diffuse and severe cerebral lesions. It is characterized by tachycardia, tachypnea, hypertension, sweating and dystonia. Usually PAID debuts in the coma-remission period, when the patient is without, or on the way out of sedation, which are days up to a few weeks after the cerebral injury. PAID may cause systemic effects, joint contractures and a severe weight loss due to the loss of muscle protein. Many of the patients who have survived cardiac arrest, but have acquired a severe, diffuse brain injury, develop autonomic dysfunction. These patients can be treated with Baclofen. It is therefore important to distinguish between generalized seizures and myoclonus caused by autonomic dysfunction. Occurrence of PAID in patients with heart failure and its impact on the cerebral outcome is not yet known (39). Currently PAID is treated with further sedation, analgesia and intrathecal baclofen. The last treatment is the one to prefer, since sedation is more a palliative treatment. Thus, when sedated, the patient will still have autonomic dysfunctions. At the same time, prolonged sedation causes secondary complications in the form of: bacteremia, pneumonia (if there is a need for mechanical

ventilation), immobility / inactivity and medication-related problems (accumulation, tolerance, withdrawal symptoms, circulatory problems and mental problems).

-Biomarkers

A number of biochemical markers have been shown to have a higher predictive value than many clinical parameters in relation to evaluation of the long-term neurological outcome in patients with cardiac arrest. These are neuron-specific enolase (NSE, gamma isomer enolase found in neurons and neuroectodermal cells), S100B (potassium-binding astroglia cell protein) and brain natriuretic peptide (BNP) (37,40 - 42). The results from the individual studies are unfortunately conflicting with respect to the importance and significance of each biochemical marker. The same applies to the time-related changes in the serum concentration versus the hypothermia treatment and "cut-off" values. It is therefore relevant to determine the sensitivity, specificity and predictive value, and to define "cut-off" level in relation to cognitive deficits in these tests. It is first and foremost concerning the NSE, S-100B, GDP and possibly the Procalcitonin (PCT) as well (31). In addition to this we wish to investigate another promising marker, CoPeptin, which is the C-terminal of pro-vasopressin. The marker can provide insight into the body's stress response, and is shown to have potential in the prognostication following cardiac arrest.

-NIRS

Near infrared spectroscopy (NIRS) is a method for determining the oxygen level in the body tissue (43). When applied to the skull, the method is used to determine the amount or fraction of oxygenated hemoglobin in the brain tissue. Studies have shown, that cerebral desaturation is a predictor of cognitive dysfunction and longer hospitalization following cardiac surgery (44). What significance the NIRS monitoring has to hypothermic treatment after cardiac arrest is however unclear. A single animal study has examined the use of NIRS in relation to cardiac arrest (45), but there are no human studies related to cardiac arrest and no studies related to therapeutic hypothermia either.

-EEG

Flat EEG during hypothermic treatment after cardiac arrest is not related to poor cerebral outcome (46). General suppression and general epileptiform activity is however (46). EEG at normothermic status following hypothermic treatment is also a strong predictor of cerebral outcome (46,47). EEG can also reveal status epilepticus without muscular activity or regular status epilepticus during treatment with neuromuscular blocking agents (48). It is not documented, whether or not recognition and treatment of status epilepticus during hypothermia, improves cerebral outcome. The recording of a standard EEG requires additional resources. Portable devices, which continuously records an EEG is easy to handle, but the applicability with hypothermia therapy is not properly validated.

Standard EEG is valuable in prediction of good as well as poor outcome, but it cannot stand alone. Other studies, which can be performed while recording the EEG, could provide additional knowledge concerning the prognosis of the patients.

BAEP (Brain Auditory Evoked Potential) is generated in the brainstem in response to a sound stimulus and reflects the functioning of the auditory system between the ear and the brainstem. The presence of BAEP has shown potential for being a positive predictor of good outcome in cases with traumatic brain injuries. In addition to this we will use the BAEP to evaluate if the patient's auditory system works, so we can rule out hearing loss as a reason for no response when performing ERP (Event Related Potential) studies as described below.

ERP, which are electrical oscillations in the cortex, can be measured by standard EEG and has shown potential as a predictor of good outcome in cases with cardiac arrest. ERP reflects the reception and further cognitive processing of external stimuli. At specific time points following a stimulus, specific ERPs occurs, which can be related to different functions of the cerebral cortex. Mismatch negativity (MMN) and P300 are two kinds of specific ERPs.

A MMN potential is triggered by a change in an otherwise continuous sound stimulus and reflects the ability to discriminate between different auditory stimuli. The presence of the MMN-potential is interpreted as the brain's automatic processing of the surrounding sounds, and reveals whether or not this ability/processing is still present following a damaging event. A P300-potential is used in multiple modalities, often auditory, and is triggered following an actionable stimulus. It reflects cognitive processes such as evaluation, categorization and selection of an appropriate response to the incoming stimulus. If these brain functions are intact, a reproducible P300 potential is triggered.

Hypothermia and exposure of the organism

As mentioned before, hypothermia has a great influence on the whole organism and its homeostasis. Slowly the patient loses consciousness. The peripheral resistance increases. ECG changes. The blood pressure and heart rate decrease, and the risk of malignant arrhythmia increases. The perfusion of individual organs decreases. Hyperglycemia occurs, the pH rises and S-potassium decreases. Hypovolemia is caused by cold diuresis and fluid extraction from the vascular bed. The muscles become stiff and metabolism decreases (49).

-Inflammatory response, oxidative stress and endothelial cell activation.

Cooling and heating of the organism cause ischemia and reperfusion-phenomenon's in different parts of the body. Ischemia-reperfusion induces a very strong activation of the immune system. The severity of this activation can be determined by measuring the inflammatory mediators like cytokines, (especially interleukin 6, 8 and 10), which are correlated with the 30-day mortality. These are measured by multiplex technique, which is a method where several mediators can be analyzed simultaneously (50). Ischemia-reperfusion events activate endothelial cells and induce the up regulation of adhesion molecules (VCAM, ICAM, and P-selectin) (51-53). Activation of the adhesion molecules is important for the interaction between the tissue and the circulating leukocytes. Part of the damage seen in the presence of ischemia-reperfusion, is due to oxidative stress, in which reactive oxygen species (ROS) cause damage to cell membranes and DNA (54). The severity of oxidative stress may be determined by measuring malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) in the blood.

-Coagulation

It is well known that a reduced temperature results in coagulopathy. Trombelastografic studies on whole blood show that hypothermia progressively weakens the coagulation system (55). Enzyme reactions are weakened and decrease by 7% for each degree the body temperature drops. Hypothermia inhibits primarily initiation of thrombin formation and inhibits fibrinogen synthesis (56). Platelet activation and aggregation are inhibited, and hypothermia will at the same time inhibit the vascular contraction when bleeding.

-Electrolytes and fluid

It is furthermore well known that hypothermia offsets the original balance of body fluids and electrolytes. Potassium migrates into the cell and fluid leaves the vascular bed.

-Kolloid osmotic pressure

The colloid osmotic pressure (CP) is an important factor in the balance of fluid between the vascular bed and the interstitial space. CP is effected of the colloids in the blood (plasma proteins) and has the function of "sucking" the filtered out fluid back to the vascular bed. An imbalance in this mechanism can cause edema (57). Therefore CP measurements have a significant importance in fluid therapy when handling intensive care patients. The method has not yet been implemented in the daily clinic, partly because of methodological problems. In pig studies however, it has been shown that CP decreases when the pigs are given hypothermic treatment (58).

-Circulation

The primarily injured organ is the heart (acute myocardial infarction or malignant arrhythmias). Practically all patients will undergo coronary angiography (CAG). It is possible that the two treatment algorithms (hypothermia for 24 or 48 hours) have different impact on circulation and a possible myocardial infarction. In the acute phase the cardiac function is primarily measured using biochemical cardiac markers, echocardiography, medication needs and continuous telemetry.

Genetics

There have been identified several genes which seem to have an influence on the effects following traumatic brain injury (rehabilitation outcome, the risk of epilepsy and cognitive deficits). Of most importance is the gene APOE. APOE is associated with increased amyloid sediments, which are expected to have an effect on the cholinergic system, oxidative stress, neuronal protection and neuronal plasticity. Since similar mechanisms probably are involved following an anoxic brain injury, it is conceivable that the presence of APOE increases the risk of cognitive damage after a cardiac arrest.

Aim

To investigate, whether treatment of cardiac arrest patients with 24 or 48 hours of mild therapeutic hypothermia gives a difference between the groups in CPC 6 months after the event.

Hypothesis

Cooling for 48 hours results in a cerebral outcome, measured by CPC, which is better than the outcome when cooling for 24 hours.

Primary outcome

CPC at 6 months following cardiac arrest. The primary outcome of the study is analyzed as the proportion of patients with good neurological outcome in the two groups, (CPC 1 and 2) at 6 month after CA.

Power calculation

The percentage of patients with a good cerebral outcome (CPC 1 or 2) following hypothermic treatment varies from study to study. In the two randomized studies from 2002, it was respectively 49% and 55%. In a multicenter study comprising 986 patients it was 46% (23).

Based on clinical considerations it is presumed, that a difference in the success rate of 15% or more between the two cooling regimes will be of clinical relevance. In the power calculation for this study the success rate of the 24 hour group is set to 50%; meaning that 50% of the enrolled patients end up with a CPC score of 1 or 2 at the time of six months following treatment. When using the equation for comparing two rates, we get that 169 patients in each group is needed to show a difference of 15% between the two groups. That is to say a success rate of the 48 hours group being $\geq 65\%$ or $\leq 35\%$. The power is set to 80% and the significance level to 5%. The calculation of power is made in collaboration with biostatisticians. The calculation of power does not take any “drop out” patients into account. To compensate for this, the total number of patients in the study is increased to 350. If the dropout rate of patients rises, this figure must be corrected so the study can include at least 338 evaluable patients.

Design

Multicenter, randomized, controlled, partially blinded, clinical study.

Randomization

The centers will have access to web-based randomization (Trial Partner). Each patient will get their own number of randomization. Randomization will take place in blocks and get stratified for center, age (above and below 60 years), and initial rhythm (shockable or not shockable rhythm). Age and initial rhythm are strong predictors of cerebral outcome when using hypothermic therapy following cardiac arrest (23).

All cardiac arrest patients that are admitted to the individual intensive care units at the different centers are screened and registered for the sake of description of the source population in the study. The reason for cooled patients, not included in the study, is registered. An example could be the lack of restoring circulation within the specified time frame, etc. (See Table 1 Action Card).

Blinding

The registration and measurement of CPC at six month will be done by research staffs that are unaware of which intervention group the patient belongs to. Registration of side effects and GCS scores while the patient is in the intensive care department will be performed by the treating

physician team and research staff, which is not blinded. The attending staff cannot be blinded in relation to which of the two intervention groups the patient belongs to. Therefore situations will not occur, where the randomization must be broken. Information concerning which intervention group the patient belongs to will be limited down to the individuals in the treatment group. Relatives and patients will not be blinded to the intervention.

Statistics, data handling and reporting

Outcome measures will be compared for the two intervention groups. The plan of data management will be laid out in collaboration with biostatisticians at the end of data collection for the basic study. Plans for interim analyses is described in the Charter for the independent Data Monitoring and Safety Committee (DMSC) (Appendix 7) .

The data is kept for 15 years after completion of data collection for the basic study and hereafter destroyed.

Data is made accessible on an international open database (ClinicalTrials.gov).

Each year throughout the trial the principal investigator must submit a list to the ethical committee of all serious expected / unexpected adverse side effects and all serious adverse events, which have occurred during the period. This information must be accompanied by an assessment of the subjects' safety.

Method

The study is divided into a basic study involving centers in Aarhus, Odense, Aalborg, Stavanger, Helsinki, Tallinn Brussels, Berlin and Turku, and additional studies, which are established in the Aarhus center in cooperation with Hammel Neurocenter. Elements from the additional studies will also be possible to participate in at other centers.

Basic study

The basic study includes:

- 1) 24 or 48 hour treatment with therapeutic hypothermia at 33 degrees Celsius
- 2) Documented standard intensive care and monitoring
- 3) Documented additional examination and treatment, including CAG and PCI
- 4) Monitoring of basic outcome parameters

Effect parameters

Primary effect parameters:

-CPC 6 months after the initiation of therapeutic hypothermia.

Secondary effect parameters:

Mortality, CPC 5 at 6 month

-Side effect frequency and severity will be followed on a daily basis, and registered at day 4, alternative at discharge from ICU and when discharged from the primary hospital.

-CPC at discharge from primary hospital unit and after 28 days plus after 3 and 6 months.

-GCS at day 4 and at discharge from primary hospital unit.

Tertiary effect parameters

-Progression of GCS throughout the observation period.

See table 2, flow sheet for basic outcome parameters

The basic study and gathering of basic effect parameters will be identical for all participating centres.

Additional studies:

The additional studies are described elsewhere, but solely include extended monitoring without additional intervention of the treatment process, and will, for some of the studies, be in the nature of quality assurance- and pilot studies. The number of patients included in these studies will be less than in the basic study. The purpose of the extended monitoring is mostly to clarify any possible connection between the measured parameters and outcomes.

Inclusion criteria

Cardiac arrest outside the hospital environment is a critical acute situation, in which documenting the exact course of time and vital parameters can be impossible to conduct. The assessment of inclusion parameters can therefore sometimes depend on the best estimate possible and verbal delivery of information from the doctor's ambulance.

- Out of hospital cardiac arrest with a presumed cardiac cause
- Re-established circulation after resuscitation (when CPR isn't necessary for 20 minutes and there are clinical signs of circulation)
- Glasgow Coma Score (GCS) < 8
- Age \geq 18 years and < 80 years

Exclusion criteria

- Estimated time interval > 60 minutes from collapse to acceptable circulation.
- Cardiac arrests with presumed non-cardiac cause (trauma, aorta dissection, intracerebral haemorrhage, stroke, massive haemorrhage or hypoxia).
- Cardiac arrest at the hospital.
- Terminal illness.
- Coagulopathy (medical anticoagulant therapy, including thrombolysis, is not a contraindication).
- Unwitnessed asystole.
- Time from cardiac arrest to initiation of therapeutic hypothermia > 240 minutes.
- An alert patient with GCS \geq 8.
- Pregnancy.
- Persistent cardiogenic shock, systolic blood pressure < 80 mmHg despite vasoactive treatment and aortic balloon pump intervention.
- Cerebral performance category (CPC) 3-4 before cardiac arrest.
- Suspected or confirmed acute intracerebral haemorrhage.
- Suspected or confirmed acute stroke.

- Acute CABG.
- Lack of consent from next of kin or family doctor / medical officer of health.
- Lack of consent from the patient if he/she wakes up and is alert.

The inclusion criteria include, as opposed to the original "evidence supporting" studies, not only patients with shockable rhythm, but also patients with non-shockable rhythm. It is estimated that this will not reduce the effect of hypothermia, perhaps the contrary.

Process of trial

Patients with out of hospital cardiac arrest usually go directly to the cardiac intervention unit to undergo coronary angiography (CAG) and possible treatment. Baseline data is recorded as early as possible. Patients who meet the inclusion criteria for therapeutic hypothermia treatment are then transferred to the ICU. In cases where the cause of the cardiac arrest appears not to be of cardiac genesis, and the patient is still comatose, the patient will undergo a CT of the cerebrum to exclude significant intracranial pathology prior to the therapeutic hypothermia.

Informed consent and randomization to 24 or 48 hours of therapeutic hypothermia must take place within the first 23 hours after the target temperature is reached.

The therapeutic hypothermia must be initiated as quickly as possible. It is desirable that the cooling starts within 60 minutes after the cardiac arrest. The cooling process starts in the cardiology intervention unit.

The cooling takes place by means of surface or I.V. cooling, possibly a combination. Adjuvant cooling treatment is administered to all patients as an infusion of NaCl of 4° C, 30 ml/kg; maximum infusion rate 100 ml/min. through large peripheral I.V. ports. Exceptions from this can be made in patients with severe cardiogenic shock or in patients undergoing dialysis. Target temperature is 33° C ± 1° C measured in the bladder or nasopharynx. Target temperature is reached when the temp. ≤ 34° C.

Temperature measurement on arrival at the hospital is carried out as a bilateral membrana tympani temperature measurement and/or via a temperature sensor, measuring in the nasopharynx.

Insertion of arterial cannula, central venous catheter and naso-gastric tube can take place at the cardiac intervention unit. If I.V. cooling is decided upon, the cooling catheter can also be inserted at the cardiac intervention unit.

Moreover, the project leaders will continuously promote and monitor the strict compliance of the treatment through their daily work with patients.

Standard intensive care treatment and cooling treatment outside the ICU, see appendix 3

Rewarming

After 24 or 48 hours of hypothermia at temperature $33 \pm 1^\circ \text{C}$, the rewarming process begins, with 0.5 degrees pr. hour until 37°C is reached. For pain relief and temperature regulation, the patient is given paracetamol $1 \text{ g} \times 4 \text{ i.v.}$, via the NStube or orally, when he/she is awake.

When the patient is warm, the sedation is stopped, with the purpose of awakening the patient and decreasing the amount of respiratory therapy needed. The patients are extubated when they meet the standard conditions for extubation. Deviation from this procedure must be justified and registered in the medical record. After being rewarmed to 37°C and extubated, the patients are observed for at least 24 hours in the ICU.

Further treatment is carried out according to the guidelines of the specific centre and on an individual basis.

Termination of active treatment

All active treatment is continued until 72 hours after normothermia has been reached. Exceptions to this may include patients who develop signs of brain death or refractory shock with multi-organ failure. It can also include patients with unrecognized terminal cancer at the initiation of the treatment, in which case further treatment is unethical. It is the medical personnel who make these considerations, independent of the research team.

Patients still comatose after 96 hours are evaluated with SSEP and a neurological assessment. The decision to terminate the intensive treatment follows international standards agreed upon by the participating centres (appendix 4), and the reason for the termination is registered in the medical record and the eCRF.

Cessation of therapeutic hypothermia treatment

There is a theoretical possibility that the cooling treatment may cause uncontrolled haemorrhage, life-threatening arrhythmias or refractory cardiogenic shock. If one of these highly rare complications should occur, the cooling treatment is terminated. The patient continues in the study and is kept at 36°C throughout the intervention period. Data is analyzed according to the “intention-to-treat” principle, meaning that monitoring and the collection of outcome data is continued throughout the whole study period, as if no parts of the treatment were changed. Data is included and analysed in the group to which the patient was allocated.

Lacking inclusion of patients

If the recruitment of subjects is slower than expected, the steering committee should consider extending the duration of the project or seek to include additional centres. With the current centres, it is expected that the inclusion of patients will take maximum 2 ½ - 3 years.

Monitoring

Standard ICU monitoring

Extended monitoring:

Basic outcome parameters are monitored in all centres

CPC, GCS, and side effects

CPC and GCS

CPC is measured at discharge of the primary hospital unit (in this case, Hammel Neurocenter is not considered a primary hospital unit), at day 28 plus after 3 and 6 months. A guideline to the CPC scoring is developed (appendix 5). If the patient is transferred to another hospital, the measurement can be conducted by telephone. For blinding see this section

GCS is measured 8 hours after the patient has been rewarmed. In addition, GCS is measured every day as long as the patient is admitted to ICUs, and measured at discharge from the primary hospital ward as well. If the patient is kept sedated, the measurement is meaningless and is therefore declared "sedated" in the data collection form.

If the patient is discharged from a hospital, the measurements are conducted by telephone.

Measuring requires communication with the wards, and the development of guidelines for the nurses in the medical wards. If it is not attempted to wake the patient when the temperature is 37° C after being rewarmed, the reason for not attempting must be indicated, also on the following days, if the patient is not awakened.

Side effects

The following side effects are registered: Pneumonia, sepsis, bleeding and coagulation, pancreatitis, electrolyte disorders and metabolic disorders, kidney failure, liver failure, pulmonary edema, seizures, arrhythmias, circulatory failure and shivering (appendix 2). The adverse events will be classified according to severity and relationship to the intervention treatment. Moreover, the various body functions are followed closely, both clinically and para-clinically, through the standard monitoring, in which the 24 hour group will act as a form of reference-/control group compared to the 48 hour group.

Side effect frequency and severity will be followed on a daily basis, and registered at day 4, alternative at discharge from ICU and when discharged from the primary hospital. For details see the Chart record form. For blinding see this section.

As the patient suffers from a severe illness that led to the cardiac arrest, serious and sometimes fatal complications will often occur during the course of treatment.

Additional outcome parameters optional for each center

Cerebral

- Somato-sensory evoked potentials (SSEP):

The right and left median nerve is stimulated with a bipolar surface stimulator. Stimulation strength is 2 x motoric threshold and the stimulation width is 200 uS. The stimulation frequency is 3 Hz. 1024 signals are collected. The signals are collected at Erbs point (brachial plexus), C7, and from the cerebral cortex (C3 'and C4'). N20 is determined, but also later occurring peaks in the response of N35 and N70 are determined.

SSEP examinations are carried out 3 times during the process, after achieving target temperature. Lack of SSEP in patients with normal temperature after 96 hours will be known by the treating physicians, and this knowledge will be applied when deciding whether to terminate the intensive care. SSEP is categorized as: 1) present bilaterally 2) present unilaterally; 3) lacking bilaterally.

-FIM (functional independent measure)

The test is performed by telephone after 3 months.

-Cognitive function

All CPC 1-3 patients are scored with MMSE and Addenbrooke's Cognitive Examination (ACE) at 6 month control. If the patients are unable to complete the examination, the reason for this is indicated on the data collection form.

-Advanced Cognitive test

An advanced cognitive test is performed on patients who can participate in the examination. It is performed 6 months after the collapse. If there are patients who cannot complete the examination, then the cause for this is registered.

Cognitive sum score. The following 5 cognitive domains are scored, and they each contribute equally to 20% of the total sum score.

Response inhibition (SCWT Interference Score)

 Visuospatial perception and construction (RCFT Copy)

 Working memory (WAIS-III)

 Processing speed (WAIS-III, TMA og TMB)

 Learning and memory, visuelt og verbalt (RCFT og RAVL)

Additionally, estimated intelligence is examined (Verbal IQ: WAIS-III subtest) but is not included as a direct effect parameter

The results from these tests and additional neuropsychological studies are part of a psychologist PhD study.

Patients who do not achieve CPC score 1 or 2 undergo a risk assessment and are examined as soon as possible at Hammel Neuro Center, according to a further described procedure. Due to logistic and economic reasons, this follow-up only includes patients at Aarhus University Hospital, Skejby.

-Cerebral and cardiac biochemical markers

NSE, CoPeptin, S100B, and NT-proBNP are measured 24, 48 and 72 hours after the target temperature has been reached. In the case of NT-proBNP, measurements are furthermore performed after 96 hours.

In the case of NSE and S100B, measurements are also performed at the patient's admission to the hospital before randomization and informed consent is achieved. We consider the measurements as validation/quality assurance of clinically used parameters. The measurements are therefore not subject to the committee law regarding the necessity of informed consent. It is known that cerebral biomarkers can be used for prognostics and for informing the next of kin. The methods are not

widely used at Aarhus University Hospital and therefore not validated and quality assured in local context. However, they will become so throughout this study.

-NIRS

Monitoring using cerebral oximetry (INVOS 5100C ®) is carried out. Monitoring starts on admission to the ICU and continues at least until the patient is awake or for 3 days. If possible, monitoring is continued for as long as the patient is unconscious and hospitalized in the ICU.

-EEG

Monitoring using continuous EEG can be carried out (using CSM M3, CSM neuro Screen ®). Monitoring starts on arrival to the ICU and continues at least until the patient is awake or for 3 days. If possible, monitoring continues for as long as the patient is unconscious and hospitalized in intensive care. Standard EEG examinations are carried out 3-4 times during the admission. In connection with the recording of EEG, we perform measurements of Event-Related Potentials (ERP), Brain auditory evoked potentials (BAEP) plus electromyographic (EMG) activity in accordance with motion commands, to see if these separately or together with the EEG can be related to the degree of brain damage, and contribute to a better prognosis of comatose patients after cardiac arrest.

-Long-term follow-up

Questionnaire (performed on all patients)

SF-12 (version 2) (generic form on self-rated health)

WHODAS-II (generic: activity / participation)

Neuro Specific questions: EBIQ/SCL-92 scales ANX, DEP and 8 (anxiety, depression and negative affect in general)

Cognitive Failures Questionnaire (Subjective cognitive complaints)

Perceived Stress Scale (general stress-strain)

Questionnaires are dispatched after 6 months 1-5 years and after 10 years.

Circulatory:

Echocardiography

Before cooling, a full cardiac ECHO.

In addition, the anaesthesiologist performs advanced FATE once a day for the first four days. All sections are stored.

Stress Response

After informed consent is obtained, blood samples are taken 3 times during the hospitalization to measure the patient's stress response.

Coagulation:

After informed consent is obtained, blood samples are taken 3 times during the hospitalization to measure dynamic and static coagulation analyses.

Fluid balance

Colloid osmotic pressure and osmolality is measured 3 times during the hospitalization but not before informed consent is obtained.

Impedance

Bioimpedance measurements are conducted while blood samples are analyzed for colloid osmotic pressure; 3 times during hospitalization, but not before informed consent is obtained.

Electrolytes

Levels of potassium, calcium, magnesium and phosphate are measured in blood and urine. Urine is collected daily for analysis of renal function and electrolytes 4 times in the process.

Other

-Blood test for genetic determination

The sample is taken during hospitalization. A detailed characterization and analysis will be described later in cooperation with the Department of Human Genetics.

-Biobank

Blood is taken for the biobank 4 times during the hospitalization, but not before informed consent is obtained. There is currently a lot of focus on the assessment of cerebral biomarkers, but many are not validated yet. If markers are developed with new and better properties than the present, it will be useful to assess them subsequently in the context of this study.

After completion of the study, the samples are frozen and stored for 5 years, after which they are destroyed.

Withdrawal of consent

Patients who wake up will be informed in writing and verbally about the study they participate in, and asked to continue in the study. If the patient refuses, the patient will be asked for the permission to use the data collected before the withdrawal of their consent and for the permission to collect primary data, i.e. CPC after 6 months.

Screening

Screening follows the CONSORT Statement. All comatose out of hospital cardiac arrest patients entering in the ICU has to be screened and registered.

Data sampling

Data collection follows the Utstein protocol (59). Data is entered online in an internet-based database (Trial Partner). Data is collected from hospital records, ambulance records and national databases. It is assumed, therefore, that the research staffs have access to these data sources, including the electronic medical record (EMR).

As long as the patients are admitted to the ICU's, each centre is responsible for: a) Screening and registration of all out of hospital comatose cardiac arrest patients b) randomization, c) treatment d) collection and entry of data according to the study protocol and the eCRF e) obtaining informed consent.

Evaluation after discharge from ICU's, is also performed by each centre, unless otherwise agreed with the Aarhus centre.

Timeline

The study will start in January 2013. From the time the first patient is randomized until the last patient is included, it will take approx. 3 years.

GCP

The study will not be GCP monitored, but will be conducted in accordance with the present protocol, the ICH-GCP guidelines and the regulatory requirements that apply to the study.

Ph.D. projects

In connection to the study, 3 additional sub studies are planned as Ph.D. projects at the center in Aarhus

Principal investigator

Hans Kirkegaard

Steering Committee

Hans Kirkegaard (chairman), Joergen Feldbaek Nielsen, Susanne Ilkaer, Anne Kaltoft, Inge de Haas, Bodil Rasmussen, Henrik Schmidt, Eldar Soereide, Alf Inge Larsen, Markus Skrifvars, Ville Pettilä, Valdo Toome, : Fabio Silvio Taccone, Christian Storm and Timo Laitio.

Participants in the project

Aarhus University Hospital, Skejby: Hans Kirkegaard, Susanne Ilkaer, Else Tønnesen, Erik Sloth, Reinhold Jensen, Anders Grejs, Christophe Duez, Anni Noergaard Jeppesen, Anne Helledie Larsen, Linda Aagaard Rasmussen, Kristoffer Noehr, Christoffer Soelling, Aage Christiansen, Jakob Gjedsted, Steven Brantlow. Anne Kaltoft, Jens Flensted Lassen, Birger Johnsen, Anne Mette Hvas Soeren Ladefoged, Anne Dilani Schroeder and Anders Boerglum.

Hammel Neurocenter: Joergen Feldbaek Nielsen, Simon Vistisen and Asger Pedersen.

Time-differentiated therapeutic hypothermia, version 6.4, March 28, 2016.

Aalborg University Hospital: Inge de Haas and Bodil Rasmussen

Odense University Hospital: Henrik Schmidt

Copenhagen University Hospital, Rigshospitalet: Christian Hassager and Jesper Kjaergaard.

Stavanger University Hospital: Eldar Soereide, Alf Inge Larsen, Kristian Strand

Helsinki: Markus Skrifvars, Ville Pettilä

Tallinn: Valdo Toome

Brussels: Fabio Silvio Taccone

Berlin: Christian Storm

Turku: Timo Laitio

Meetings

The steering committee will meet/communicate regularly.

Data Monitoring and Safety Committee (DMSC)

Anders Perner, chairman (Copenhagen), Kjetil Sunde (Oslo), Michael Haney (Umeaa) and

Risto Roine (Turku)

The obligations of the DMSC are described in a charter in appendix 7.

Publications

Authorships will follow the Vancouver convention. Hans Kirkegaard will be the first author of the primary outcome study. The Ph.D. student will be the first author of the respective papers that evolves from the Ph.D. sub studies.

Economy

The basic costs to run the study are outlined in the budget. There will continuously be fund rising activities in order to secure the economy of the study. When the basic costs of the study are covered, the incoming funds will be distributed among the centers according to the number of patients they include in the study.

Ethics

Mild therapeutic hypothermia is an evidence based treatment of comatose out of hospital cardiac arrest victims. Today cooling to 33±1 °C for 24 hours is standard treatment in great parts of Europe. The treatment results in better cerebral outcome and reduced mortality. However there is still a lot of unanswered questions concerning the treatment. One of the questions addressed in the present study is: for how long time should the hypothermia treatment be continued? In the participating centers in this study, standard duration is 24 hours. However, it is unknown, if prolongation of mild hypothermia for more than 24 hours could improve cerebral outcome even more. Newborns, who are suffering from perinatal hypoxia and possible cerebral damage benefit from hypothermia treatment for 72 hours (18). It is therefore reasonable to believe that adults also might benefit from prolonged therapeutic hypothermia. Prolongation of the treatment could however increase the risk of infection, but at the same time also increase the possibility of a better cerebral outcome. As the cerebral outcome is the overall dominating factor for a successful treatment of out of hospital cardiac arrest, we see no ethical problems with a treatment group of 48 hours of mild therapeutic hypothermia. The theoretical possibilities of improved cerebral outcome balance the possibility of an increased infection rate. Recently a multicenter study examined 24 hours of hypothermia at 33 °C versus 24 hours at 36 °C. The study showed no difference between the two temperatures in cerebral outcome or adverse events (60). However, the international guidelines still recommend that clinicians provide post resuscitation care based on the current treatment recommendations (33°C).

The study includes unconscious patients that are unable to communicate with the investigator. Accordingly, it carries special challenges and considerations to obtain the informed consent, and the procedure has to be adapted to the rules and bylaws in the different countries participating in the study.

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Table 1

Action Card

	Admission Hospital	Admission ICU	Day 1	Day 2-4	Day 5 to discharge	Day 28	3 month	6 month
Screening		X						
Randomizing		X						
Temperature	X	X	X	X	X			
Cooling	X	X	X	X				
Baseline Data	X	X						
Standard monitoring.	X	X	X	X	X			
Standard treatment	X	X	X	X	X			
Standard blood samples.	X	X	X	X	X			
NSE og S100B Optional	X	X	X	X	X			
Examinations	KAG? CTC?							
Treatment	PCI?							
Rewarming				X				
Stop sedation				X				
Outcome parameters	X	X	X	X	X	X	X	X

Table 2 Outcome parameters and Flow Sheet

Day	Time	CPC	GCS	Adverse events
0	T0		X	X
1	T24			X
2 - 3	Normotherm +8		X	X
2	48		X	X
3	72		X	X
4	96		X	X
5			X	X
6			X	X
7			X	X
Discharge ICU			X	X
Discharge primary hosp.		X		X
1 month		X		
3 month		X		
6 month		X		

CPC: Cerebral Performance Category Score

GCS: Glasgow Coma Score

Appendix 1

Classification of events and complications

Description of side effects/complications

Side effects/complications should be described; length of time and severity should be noted, as well as its relation to the therapeutic hypothermia, treatment and outcome. All side effects should be registered in the e-CRF from the time of admission to day 4/discharge from ICU and from day 4/discharge from ICU to discharge from hospital.

For every registered severe side effect, you will be asked whether this is a SUSAR (Suspected unexpected serious adverse reaction). A SUSAR is a serious side effect, and the nature of the effect as well as the severity has not been described in the literature of therapeutic hypothermia side effects.

Severity

Mild: Transient symptoms, no interference with organ function, acceptable.

Moderate: Distinct symptoms, moderate interference with organ function but still tolerable.

Severe: Considerable interference with organ function, unacceptable. Examples: uncontrollable bleeding, intracranial bleeding, septic shock, life threatening arrhythmias in need of CPR.

The side effect and its relation to the therapeutic hypothermia

Probable: A good explanation and sufficient documentation demonstrating a causal relationship.

Possible: A causal relationship is possible and cannot be ruled out.

Unlikely. The event is probably related to another etiology than the therapeutic hypothermia.

Unknown: The relationship cannot be determined due to poor documentation or conflicting data.

Side effects are treated according to the local standard operating procedure and should be followed until the side effect is over or until the condition has stabilized.

Appendix 2

Events and complication registration

Events are collected from s and registered at three occasions

- 1) FROM: admission - TO: end-of-Day-4 or Discharge (ICU)
- 2) FROM: Day 4/Discharge (ICU) - TO: discharge (prim. hospital)
- 3) If the patient die in-hospital.

Cerebral

Pupils: reacting to light, dilated (size), side difference?

Seizure: involuntary contractions or series of contractions of the voluntary muscles.

Myoclonus: short lasting involuntary contractions of one or several muscles

Myoclonus state: continuous myoclonus.

Convulsive State: continuous seizures or continuous seizure pattern on EEG

Circulation

Hypotension

Mild: Mean arterial pressure (MAP) > 60 mmHg with one inotropic and volume

Moderate: MAP > 60 mmHg with full treatment

Severe: MAP 50 – 60 mmHG despite full treatment

Circulatory failure: MAP < 50 mmHg for more than 10 min despite full treatment.

Need for pacing.

Need for Cardiopulmonary resuscitation.

Arythmias (y/n)

Mild: arrhythmias that do not demand treatment

Moderate: stable hemodynamics (MAP >60) with treatment

Severe: VT/VF or unstable hemodynamics despite treatment

Gastrointestinal

Mild: Aspiration, can partly take enteral nutrition

Moderate: Aspiration more than 400 ml cannot take any enteral nutrition

Severe: Ileus, bleeding gastric ulcer, need for explorative laparotomy, others specify

Urologic

Dialyses: Continuous veno-venous haemodiafiltration or intermittent haemodialyses

Infectious/inflammatory

Pneumoni: The diagnose is based on the following criteria's

1. New or progressing infiltrations on thorax x-ray.
2. Fever (not during hypothermia treatment)
3. Leukocytosis
4. Purulent tracheobronchial secretion

SIRS: The Systemic Inflammatory Response Syndrome (SIRS) is present, when two of the following 4 abnormalities is present (not during hypothermia treatment).

Temperature > 38 °C or < 36 °C.

Pulse rate > 90 beats/min.

Respiration rate > 20 breath/min or PaCO₂ < 4.3 kPa or need for mechanical ventilation.

Leucocytes > 12000 cells/cubic millimeter, or < 4000 cells/cubic millimeter, or > 10% immature cells.

Sepsis: Sepsis is SIRS caused by an infection.

Severe sepsis: Is sepsis associated with organ dysfunction (hypo perfusion) or hypotension. Hypo perfusion and perfusion abnormalities can include, but are not restricted to, lactate acidosis, oliguria or an acute change in mental status.

Septic shock: Is sepsis with hypotension (systolic blood pressure < 90 mm Hg or a reduction > 40 mm Hg from baseline) and perfusions abnormalities or need for vasoactive drugs despite adequate volume treatment in the absence of other reasons for hypotension.

Bleeding and transfusion

Bleeding Severity:

Mild: no transfusion needed.

Moderate: up to two SAG M units/24 hours.

Severe: more than two SAG M units/24 hours

Critical bleeding in organs: Intracranial, intraspinal, intraocular, pericardial.

Other bleeding: Retroperitoneal, thorax, solid organs.

Transfusion

Number of SAG M, FFP, thrombocyte units given

Appendix 3

Treatment and monitoring protocol

Standard of care and monitoring

TREATMENT

COOLING: Please refer to the protocol, cooling is started as soon as possible and both superficial and internal cooling can be used. Cold saline 30ml/kg is given if no contraindications exist.

CNS: The headboard is elevated to an angle of 30 degrees in order to prevent ventilator-associated pneumonia (VAP). Sedation with Propofol/Ultiva or Midazolam/Fentanyl depending on haemodynamic stability. Relaxation with Cisatracurium-infusion until 33 ± 1 °C is achieved, hereafter as needed (for example if shivering occurs). Convulsions are treated aggressively with additional sedation using Midazolam or Propofol and beyond this, the local standard operating procedure should be followed.

Respiration: Normoventilation (CO₂ 4.5 – 5.5 kPa), pressure controlled, PEEP 5 cm H₂O, SaO₂ ≥ 95%.

Circulation: The aim is sinus rhythm and a mean arterial pressure (MAP) of above 60 mmHg. If circulatory support is needed, Dopamine, Dobutamin or Noradrenalin can be used. If the circulatory failure is severe, individual treatment is necessary with Adrenaline, Milrinone and Simdax and possibly intra-aortic balloon pump. In this situation local standard operating procedures for inotropic and antiarrhythmic agents could also be used.

The primary treatment for tachyarrhythmia is Amiodaron supplemented with magnesium. Drug resistant new-onset atrial fibrillation or flutter and VT as well as VF are treated with defibrillation. If bradycardia occurs, external pace pads should be placed and internal pacing electrodes considered. Hematocrit should be ≥30.

Abdomen: Pantoloc 40 mg x 1 i.v. Enteral nutrition 10 ml/h for the first 24 hours and hereafter 20 ml/h from 24 to 48 hours. The enteral nutrition is paused when rewarming begins. No parenteral nutrition should be used. Blood glucose should be maintained between 6-8 mmol/l using glucose and insulin i.v.

Renal: Urinary catheter with temperature monitoring. The urinary output should be >1 ml/kg/hour. The primary treatment should be volume; treatment with a loop diuretic may be necessary. The aim is a positive fluid balance during the first 24 hours. Unless there are contraindications, all patients receive 30 ml/kg isotonic saline with a temperature at 4°C (100 ml/min) during the cooling process. The fluid therapy should be guided by hourly diuresis, as well as hemodynamics and echocardiography measurements.

Electrolytes: Serum potassium will decrease due to cooling because the ion is shifted intracellularly. The aim is serum potassium of 3.5-4.5 mmol/l. Treatment with potassium infusion should be started during the cooling process when serum potassium reaches 3.5 mmol/L. Prior to normal temperature levels, potassium infusion should be stopped when serum potassium reaches 4.0 mmol/L. The preferred administration route is intravenously since the uptake of oral potassium solutions can be influenced by the hypothermia therapy.

Infection: Cefuroxim 1.5 g x 3 is given to all patients as prophylaxis. Supplemental treatment is based on individual judgment.

Coagulation: Fragmin 5000 International Units x 1 subcutaneously. Supplemental treatment based on cardiology consultation.

Bleeding: Is treated according to the local standard operating procedure using blood products. The aim is a hematocrit ≥ 30 .

Fever control: Paracetamol 1 g x 4 + 1g on demand, maximum times two per day. Administration is i.v. when the patient is hypothermic, otherwise i.v or in the nasogastric tube. Treatment is at the latest initiated when rewarming is started.

Cooling outside of the intensive care unit.

In the beginning of the hospital course, the patient will occasionally be outside of the intensive care unit as part of treatment/investigation such as cardiology intervention suite, CT scanner or during transportation. The aim is to start the cooling process as soon as possible even though it is not possible to connect the patient to the long term cooling system.

In order to begin the cooling process and to prevent heating, the following methods should be used either alone or in combination.

1. Cold isotonic saline i.v. The maximum dose is 30 ml/kg
2. Undress the patients and lower the room temperature.
3. Ice or cold packs placed over the large superficial arteries.
4. The placement of 1 – 3 EMCOOL pads on the patient's body

A nasopharyngeal sensor or an infrared tympanic membrane sensor should be used to monitor the temperature.

MONITORING

CNS: Pupillary light reflex, convulsions, myoclonus, level of sedation (GCS), time to awakening following stop of sedation; sedation is evaluated using a sedation score. The presence of paresis is evaluated on the non-sedated patient. CT scan of the cerebrum is performed according to the local standard operating procedure.

Circulatory: Invasive blood pressure monitoring (radial artery), central venous pressure, daily 12 lead EKG as well as continuous 2 lead monitoring (V5 and II), arrhythmias are registered. At admission an echocardiography is performed by a cardiologist. Central venous oxygen saturation, ScvO₂ x 3/24 hours and Swan Ganz monitoring according to the local standard operating procedure. Need for inotropic, pacing or cardiopulmonary resuscitation is registered

Blood tests: Lactate, cardiac enzymes should be measured daily (TNT, CK, and CKMB).

Respiratory: Arterial blood gases every hours initially, hereafter every 4 hours as well as when needed, airway pressure, respiratory minute volume, PEEP, FiO₂, SaO₂, abnormal breath sounds. Chest x-ray at admission, hereafter on a daily basis until extubation

Gastrointestinal: Secretions, possibly bladder pressure monitoring.

Blood tests: Bilirubin, LDH, ALAT and amylase on a daily basis.

Temperature: Bladder/esophagus and peripheral (dorsal part of the foot).

Renal: hourly diuresis. Level of hydration, fluid balance, weight, clearance and electrolytes on a daily basis.

Coagulation: Bleeding. Blood tests: thrombocytes, INR, APTT on a daily basis

Infection: Petechial, Sepsis (definition appendix 2), pneumonia (definition appendix 2) and other infections, Sputum culture, urinary culture and blood culture on a daily basis. Blood test: leucocytes and C-reactive protein on a daily basis.

Blood test: The above-mentioned blood tests are performed at admission and then on a daily basis.

Other monitoring of the patient is performed based on the local standard operating procedure and based on clinical judgment.

Nursing evaluation: nursing observations and problems associated with the therapeutic hypothermia.

Appendix 4

Neuro prognostication

Prognostication of cerebral outcomes in patients with lack of awakening after rewarming is performed as described in the attached guideline elaborates by a work force under The Danish Society of Intensive Care Medicine and The Danish Society of Anaesthesiology and Intensive Care Medicine.

Appendix 5

CPC scoring

CPC stands for Cerebral Performance Category (1). It is a scoring system that evaluates the cerebral function/performance, and it is typically used in patients that have suffered from cardiac arrest.

The CPC scoring is simple and gross, as it only operate with 5 categories (see table). It primarily focuses on the patient's ability to work and to take care of him/herself.

CPC I	Good Cerebral Performance. Conscious: Alert, able to work and lead a normal life. May have minor psychological or neurological deficits (mild dysphasia, no incapacitating hemiparesis, or minor cranial nerve abnormalities).
CPC II	Moderate Cerebral Disability. Conscious. Sufficient cerebral function for part-time work in sheltered environment or independent activities of daily life (dressing, traveling by public transportation, and preparing food). May have hemiplegia, seizures, ataxia, dysarthria, dysphasia, or permanent memory or mental changes.
CPC III	Severe Cerebral Disability. Conscious. Dependent on others for daily support because of impaired brain function (in an institution or at home with exceptional family effort). At least limited cognition. Includes a wide range of cerebral abnormalities from ambulatory with severe memory disturbance or dementia precluding independent existence to paralytic and able to communicate only with eyes, as in the locked-in syndrome.
CPC IV	Coma, Vegetative State. Not conscious. Unaware of surroundings, no cognition. No verbal or psychological interactions with environment.
CPC V	Death. Certified brain dead or dead by traditional criteria.

According to their performance the patients are scoring I, II, III, IV or V. It's especially the distinction between category II and III that is challenging and especially in patients outside the labor market. The central point is to assess, if the patient is capable of taking care of himself (CPC I or II), or if he is dependent on others (CPC III or IV).

The importance of the distinction between category II and III is underlined of the fact that CPC I and II is considered as "Good outcome" and CPC III-IV is considered as "Bad outcome" when CPC scoring is used to evaluate the effect of therapeutic hypothermia following cardiac arrest .

1. Christopher M. Booth et al. Is This patient dead, vegetative. or severely neurologically impaired? Assessing outcome for comatose survivors of cardiac arrest. JAMA 2004; 291: 870-9.

Appendix 6

Collaboration and agreement template

Between

Aarhus University Hospital, Department of Anesthesiology and Intensive Care, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark and Aarhus University, Department of Clinical Medicine, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark, hereinafter together referred to as “AUH” and [institution name and address xx] hereinafter referred to as “X”.

1. Introduction

This document encompasses the research collaboration agreement concerning execution of the clinical trial “Time-differentiated treatment with mild therapeutic hypothermia, a multicenter study”

The study protocol is part of this document, and attached hereto as exhibit 1. AUH is clinical sponsor of the protocol and Prof. Hans Kirkegaard is principal investigator. The parties wish to collaborate on the protocol and conduct the study in each party respectively under the terms of this agreement and applicable law. The Parties shall comply with all applicable national and international laws, regulations and guidelines, especially those governing the conduct of clinical trials, dealings in medicinal products, responsibilities of clinical investigators, informed consents, protection and privacy of personal data and storage of data and records, including, without limitation, the ICH Guidelines and the European Guidelines on Good Clinical Practice (hereinafter referred to as “ICH-GCP”), Good Laboratory Practice, the revised versions of the Declaration of Helsinki Directive 95/46/EC and Directive 2001/20/EC of the European Parliament and of the Council, and professional industry association regulations

2. Steering Committee

The study is led by a Steering Committee consistent of representatives of the parties. The Principal investigator is chairman of the committee. Each center can have two members in the steering committee.

Each center has a local investigator that is responsible for the study at the specific center. Each center agrees that the collection, processing and disclosure of personal data and medical information related to the TTH48-study subjects, and personal data related to the center investigators and any investigational staff is subject to compliance with applicable personal data protection and security laws and regulation. Each center shall assist the principal investigator in obtaining all necessary

approvals from the national ethics committees, hereunder but not limited to the protocol and its amendments and informed consent form, and relevant regulatory authorities.

The steering committee communicates primarily by email, but every 6 month, or when needed, there will be a Skype or video meeting.

3. Data

The steering committee owns the data, which may utilize the data in any way it deems appropriate, subject to an in accordance with applicable privacy and security laws and regulations and the terms of this Agreement. But the individual centers should be allowed to use its generated center data for further non-commercial research and teaching purposes after proper publication of this multicenter study or if the steering group gives ist permission.

Notwithstanding the foregoing, each center retains ownership of all raw clinical data, including biological materials, as contained in the respective center's patient and medical records or other original source documentation.

4. Authorship

The results of the study will be jointly published in an international peer-reviewed journal. The Principal Investigator is 1st author. Each center will, per 15 patients they include and follow hold 1 authorship, and additionally 1 for every 15 patients included and followed up. However the first authorship will be relished when 5 patients are included and followed up, that is 30 patients included 2 authors, 45 patients included 3 authors etc.

Writing group. A writing group of four named persons will prepare the final manuscript

5. Termination of the study

Randomization of all 350 patients is expected to end before December 31, 2015. If this is not possible an interim analyses will be performed in the autumn 2015, when half of the patients (175) have been followed up (6 month after the cardiac arrest). Allocation of patients will continue during the interim analyses process. The continuation of the study will depend on the results of the interim analyses, and based on the recommendations from the Data and Safety Monitoring Committee.

6. Data and Safety Monitoring Committee

An independent data and safety monitoring committee (DSMC) is appointed by the TTH48 Steering Committee. The responsibilities of the committee are described in the charter for the independent DMSC of the TTH48-trial.

7. Payment

Each center will be paid 120 Euro per patient they include. Bank transfer of funding from AUH shall be made to each center by center's invoice.

8. Confidentiality

Any information acquired by a party from the other party concerning the TTH48 study, the protocol or data (hereinafter "Confidential Information") shall be treated as confidential and maintained in confidence by the parties. Confidential Information shall not be used by a party without the other parties written consent, which shall not be unreasonably withheld. The obligation of confidentiality under this Agreement shall remain in force until three (3) years after the conclusion or early termination of the TTH48-Study.

The following information is not considered Confidential Information:

- (i) Information that is already known at the time of its revelation or later is made public through no fault of one of the parties or;
- (ii) Information that a party at the time of its revelation can show by written records was already known by the party or;
- (iii) Information that a party has received in good faith from a third party through no breach of obligations of confidentiality or;
- (iv) Information that can be demonstrated as independently developed or acquired by a party without reference to or reliance upon information disclosed by the other party or;
- (v) Information that is required to be disclosed by law.

After conclusion or early termination of the TTH48-Study, the parties undertake to return all Confidential Information without undue delay. The parties are allowed to keep one (1) copy of the Confidential Information for regulatory purposes.

Where employees or co-workers of the parties necessarily have access to the Confidential Information the Parties shall impose the same obligations on them.

9. Liability and warranty

Each party will be liable for its own acts and omissions in relation to the TTH48-study and shall not be responsible for the acts and omissions of the other parties. Any obligation to pay damages does however not include indirect losses, consequential damages and operational losses, lost profit or other consequential financial losses, including claims for damages from a third party.

The parties enrolling study subjects under this agreement shall be covered by insurance or self-insurance in an amount sufficient to support its obligations and study subjects under this Agreement. AUH is as public Danish body self-insured and its assets are sufficient to cover any contemplated self-insured liability assumed under this Agreement. Study subjects enrolled in Denmark are covered by Danish mandatory law “Lov om klage- og erstatningsadgang inden for Sundhedsvæsenet, kapitel 4, (act no 547 of 24. June 2005) as amended from time to time.

10. Miscellaneous

The investigator at each centre confirms that there is no conflict of interest that will inhibit or affect the performance under of the TTH48-Study and confirm that their performance under this Agreement does not violate any other agreement with third parties .In the event of any dispute arising between the parties in relation to the terms of this agreement, the parties shall use their best endeavours to resolve the matter on an amicable basis. This agreement shall be governed by and shall be construed in accordance with the laws of Denmark without regard to any conflicts of law’s provisions. The parties consent to the competent courts of Denmark for the resolution of all disputes or controversies between the parties hereto that the parties are unable to settle amicably.

Aarhus University

[institution name and address,]

Institute for Clinical Medicine

XXXXXXXXXXXXX

_____ date_____

_____ date_____

Kristjar Skajaa, director

[Name, titel]

_____ date_____

Hans Kirkegaard, principal investigator, professor

Aarhus University Hospital

Department of Anesthesiology and Intensive Care

_____ date_____

Karsten Hindsholm

Head of Department

Appendix 7

Charter for the independent Data Monitoring and Safety Committee (DMSC) of the TTH48-trial

Clinical Trial no. NCT01689077 Research ethical committee no. 20110022, Central Denmark Region

2011

Introduction

The Charter will define the primary responsibilities of the DMSC, its relationship with other trial components, its membership, and the purpose and timing of its meetings. The Charter will also provide the procedures for ensuring confidentiality and proper communication, the statistical monitoring guidelines to be implemented by the DMSC, and an outline of the content of the Open and Closed Reports that will be provided to the DMSC.

Primary responsibilities of the DMSC

The DMSC will be responsible for safeguarding the interests of trial participants, assessing the safety and efficacy of the interventions during the trial, and for monitoring the overall conduct of the clinical trial. The DMSC will provide recommendations about stopping or continuing the trial to the Steering Committee (SC) of the TTH48 trial. To contribute to enhancing the integrity of the trial, the DMSC may also formulate recommendations relating to the selection/recruitment/retention of participants, their management, improving adherence to protocol-specified regimens and retention of participants, and the procedures for data management and quality control.

The DMSC will be advisory to the SC. The SC will be responsible for promptly reviewing the DMSC recommendations, to decide whether to continue or terminate the trial, and to determine whether amendments to the protocol or changes in trial conduct are required.

The DMSC is not planned to meet physically in order to evaluate the planned interim analysis of the TTH48-trial. The interim analysis will be performed by an independent statistician (member of the DMSC). The DMSC may whenever they decide, contact each other by telephone, Skype or e-mail in order to discuss the safety for trial participants. Sponsor has the responsibility to report yearly to the DMSC the overall number of Serious Adverse Reactions (SAR). The DMSC can at any time during the trial request the distribution of events, including outcome measures and SARs according to intervention groups. The recommendations of the DMSC regarding stopping, continuing or changing the design of the trial should be communicated without delay to the SC of the TTH48-

trial. The SC has the responsibility to inform as fast as possible, and no later than 72 hours, all investigators of the trial and the sites including patients in the trial about the recommendation of the DMSC and the SC decision hereof.

Members of the DMSC

The DMSC is an independent multidisciplinary group consisting of clinicians and a biostatistician that, collectively, has experience in the management of ICU patients and in the conduct, monitoring and analysis of randomised clinical trials.

DMSC Clinician

Anders Perner, chairman (Copenhagen)

Kjetil Sunde (Oslo)

Michael Haney (Umeaa)

Risto Roine (Turku)

A biostatistician independent of the trial will prepare and analyses the interim analyses data

Conflicts of interest

DMSC membership has been restricted to individuals free of conflicts of interest. The source of these conflicts may be financial, scientific, or regulatory in nature. Thus, neither trial investigators nor individuals employed by the sponsor, nor individuals who might have regulatory responsibilities for the trial products, are members of the DMSC.

The DMSC members will disclose to fellow members any consulting agreements or financial interests they have with the sponsor of the trial, with the contract research organization (CRO) for the trial (if any), or with other sponsors having products that are being evaluated or having products that are competitive with those being evaluated in the trial.

The DMSC will be responsible for deciding whether these consulting agreements or financial interests materially impact their objectivity.

The DMSC members will be responsible for advising fellow members of any changes in these consulting agreements and financial interests that occur during the course of the trial. Any DMSC members who develop significant conflicts of interest during the course of the trial should resign from the DMSC.

DMSC membership is to be for the duration of the clinical trial. If any members leave the DMSC during the course of the trial, the SC will appoint the replacement(s).

Safety and Interim analyses

When 175 patients are allocated an interim safety analyses based on the number of SARs (including death) during the primary hospital admission will be performed

When the main outcome score CPC at six month is measured in 175 patients an interim analysis will be performed, unless the DMSC recommend, and the SC decides otherwise.

Formal interim analysis meeting

The DMSC meetings will be by Skype or telephone and only exceptionally face to face. One 'Formal Interim Analysis' meeting will be held to review data relating to treatment efficacy, patient safety, and quality of trial conduct. The members of the DMSC will meet when 180-day follow-up data of 175 patients have been obtained.

Proper communication

To enhance the integrity and credibility of the trial, procedures will be implemented to ensure the DMSC has sole access to evolving information from the clinical trial regarding comparative results of efficacy and safety data, aggregated by treatment groups. An exception will be made to permit access to an independent statistician who will be responsible for serving as a liaison between the database and the DMSC.

At the same time, procedures will be implemented to ensure that proper communication is achieved between the DMSC and the trial investigators. To provide a forum for exchange of information among various parties who share responsibility for the successful conduct of the trial, a format for Open Sessions and Closed Sessions will be implemented. The intent of this format is to enable the DMSC to preserve confidentiality of the comparative efficacy results while at the same time providing opportunities for interaction between the DMSC and others who have valuable insights into trial-related issues.

Closed Sessions

Sessions involving only DMSC membership who generates the Closed Reports (called Closed Sessions) will be held to allow discussion of confidential data from the clinical trial, including information about the relative efficacy and safety of interventions. In order to ensure that the DMSC will be fully informed in its primary mission of safeguarding the interest of participating patients, the DMSC will be blinded in its assessment of safety and efficacy data. However, the DMSC can request unblinding from the SC.

Open Reports

For each DMSC meeting, Open Reports will be provided available to all who attend the DMSC meeting. The Reports will include data on recruitment and baseline characteristics, and pooled data on eligibility violations, completeness of follow-up, and compliance. The primary trial statistician will prepare these Open Reports.

Closed Reports

Will include analysis of the primary efficacy outcome measure. In addition, analyses of the secondary outcome measures and serious adverse events will also be reported. These Closed Reports will be prepared by an independent biostatistician, with assistance from the trial biostatisticians, in a manner that allow them to remain blinded.

The Closed Reports should provide information that is accurate, with follow-up on mortality that is complete to within two months of the date of the DMSC meeting.

The Reports should be provided to DMSC members approximately three days prior to the date of the meeting.

Minutes of the DMSC Meetings

The DMSC will prepare minutes of their meetings. The closed minutes will describe the proceedings from all sessions of the DMSC meeting, including the listing of recommendations by the committee. Because it is likely that these minutes may contain unblinded information, it is important that they are not made available to anyone outside the DMSC.

Recommendations to the Steering Committee

After the interim analysis meeting, the DMSC will make a recommendation to the SC to continue, hold or terminate the trial.

If an analysis of the interim data from 175 patients fulfils the Haybittle-Peto criterion, the inclusion of further patients will be paused and an analysis including patients randomized during the analysis and period of pausing the trial will be performed. If this second analysis also fulfils the Haybittle-Peto criterion or the group sequential monitoring boundaries the DMSC will recommend stopping the trial.

If the recommendation is to stop the trial the DSMC will discuss and recommend on whether the final decision to stop the trial will be made after the analysis of all patients included at the time (including patients randomised after patient number 175), and whether a moratorium shall take

place (setting the trial at hold) in the further inclusion of patients during these extra analyses. If further analyses of the patients included after 175 patients is recommended rules for finally recommending stopping of the trial should obey the Lan DeMets stopping boundary. This recommendation will be based primarily on safety and efficacy considerations and will be guided by statistical monitoring guidelines defined in this Charter.

The SC is jointly responsible with the DMSC for safeguarding the interests of participating patients and for the conduct of the trial. Recommendations to amend the protocol or conduct of the trial made by the DMSC will be considered and accepted or rejected by the SC. The SC will be responsible for deciding whether to continue, hold or stop the trial based on the DMSC recommendations.

The DMSC will be notified of all changes to the trial protocol or conduct. The DMSC concurrence will be sought on all substantive recommendations or changes to the protocol or trial conduct prior to their implementation.

Statistical monitoring guidelines

The outcome parameters are defined in the TTH48-trial protocol. For the two intervention groups, the DMSC will evaluate data on:

Interim safety analyses

The occurrence of SARs from start of intervention until discharge from primary hospital in the first 175 patients.

Interim analyses

The primary outcome measure: CPC at 6 month after randomization, when 175 patients have been followed up for 6 month.

Mortality at 6 month after randomization, when 175 patients have been followed up for 6 month

The DMSC will be provided with these data from the Coordinating Centre as:

Number of patients randomised

Number of patients randomised per intervention group (0.1)

Number of events, according to the primary outcomes, and occurrence of SARs including death in the two groups.

Based on evaluations of these outcomes, the DMSC will decide if they want further data from the Coordinating Centre and when next to perform analyses of the data.

For analyses, the data will be provided in one file as described below.

Safety monitoring

Based on the analyses of SARs (including death) in the first 175 patients, the DMSC will use $P < 0.001$ (Haybittle-Peto) as the statistical limit to guide its recommendations regarding early termination of the trial.

Interim analyses

Based on 6 month CPC analyses in 175 patients, the DMSC will use $P < 0.001$ (Haybittle-Peto) and group sequential monitoring boundaries as the statistical limit to guide its recommendations regarding early termination of the trial.

DMSC should also be informed about all SARs occurring in the two groups of the trial.

The DMSC may also be asked to ensure that procedures are properly implemented to adjust trial sample size or duration of follow-up to restore power, if protocol specified event rates are inaccurate. If so, the algorithm for doing this should be clearly specified.

Conditions for transfer of data from the Coordinating Centre to the DMSC

The DMSC shall be provided with the data described below in one file.

The DMSC will be provided with an Excel database containing the data defined as follows:

Row 1 contains the names of the variables (to be defined below).

Row 2 to N (where N-1 is the number of patients who have entered the trial) each contains the data of one patient.

Column 1 to p (where p is the number of variables to be defined below) each contains in row 1 the name of a variable and in the next N rows the values of this variable.

The values of the following variables should be included in the database:

- 1: PtID: a number that uniquely identifies the patient.
- 2: Rdcode: The randomisation code (group 0 or 1) – the DMSC is not to be informed on what intervention the groups received.
- 3: SARHosp: Severe Adverse Reaction from start of intervention until discharge from primary hospital (1 if patient has had SAR 0 if the patient did not).

Time-differentiated therapeutic hypothermia, version 6.4, March 28, 2016.

4: CPC6m: 6 month CPC outcome, good (CPC 1 and 2) or bad (CPC 3-5), (0 good outcome, 1 bad outcome)

5: Mor6m: 6 month mortality (0 dead, 1 alive)

April 12, 2015

Hans Kirkegaard

