Protocol for Meta-Analysis of temperature reduction in animal models of cardiac arrest
Hilmer Olai, Gustav Thornéus, Hannah Watson, Malcolm Macleod, Hans Friberg, Jonathan Rhodes, Tobias Cronberg, Niklas Nielsen, Tomas Deierborg
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• Research question

Background
Out-of-hospital cardiac arrest is common and affects more than 10 000 people in Sweden each year. Over the past 10 years, survival rates have doubled and today more than 1 000 patients each year are resuscitated and admitted to a hospital bed of whom approximately 50% are alive one month later (1). Immediately after onset of cardiac arrest, i.e. global brain ischemia, energy depletion in the brain tissue leads to a severe failure of cellular homeostasis with subsequent cell death and inflammation in the brain. Experimental animal models have shown that the body temperature after cardiac arrest has a large effect on the extent of brain damage that develop after global ischemia (2) (3) (4) (5) (6). Elevated body temperature, fever, is associated with increased damage (7) (8) while cooling, hypothermia, has a protective effect in several injury models. In 2002, two landmark randomized clinical trials testing the effects of therapeutic hypothermia in patients with cardiac arrest were published (9) (10). The studies showed improved neurological function and increased survival in patients cooled to 33 ºC for 12-24 hours compared to patients with no hypothermia treatment. These studies, together with a Cochrane review (11), have had a major impact in the international guidelines and therapeutic hypothermia is currently the recommended treatment for patients who regain circulation after cardiac arrest (10). However, the concept of therapeutic hypothermia has recently been challenged by the Target Temperature Management trial which included a large sample of cardiac arrest patients (n=939) and found no benefit from hypothermia at 33ºC as compared to controlled temperature at 36ºC on survival (12), detailed neurological outcome (13) or release of neuron-specific enolase (14). In addition, the THAPCA-OH-trial reported that therapeutic hypothermia at 33ºC after cardiac arrest in children, compared with therapeutic normothermia at 36.8ºC, did not confer any significant benefit in survival with a good functional outcome at one year (15). Another meta-analysis on clinical trials also questions the supporting evidence for therapeutic hypothermia after
cardiac arrest (16). To date there is no preclinical meta-analysis investigating the effects of temperature reduction after cardiac arrest. With this study the authors hope to explain possible translational gaps between animal models and patients and provide methodological considerations for future experimental research and clinical trials.

**Research question**
What are the effects of temperature reduction in animal models of cardiac arrest on histological outcome, neurobehavioral outcome and mortality?

**Objectives**

1. To assess the quality of individual studies investigating temperature reduction in animal models of cardiac arrest.
2. To assess the efficacy (reported as histological outcome, neurobehavioural outcome or mortality) of temperature reduction in animal models of cardiac arrest and the impact of study design factors on that efficacy.
3. To assess the range of supporting evidence using a modified STAIR-criteria.

With the results from 1-3, the authors aim to explore and discuss possible:
4. Translational gaps in the available data on the effect of temperature reduction in animal models of cardiac arrest.
5. Sources of bias and sources of heterogeneity in animal studies.
• **Inclusion & exclusion criteria**

**Inclusion**
– They have a control group.
– They induce global ischemia in the brain of an adult*, living non-human animal (mammal).
– They investigate a reduction of temperature after global ischemia.
AND
– They report a histological outcome assessment of neuronal cell death/injury in brain tissues**
  OR
– They report a neurobehavioural assessment of outcome
  OR
– They assess the mortality of the temperature reduction.

* Animals assumed adult unless manuscript says it is modeling neonatal responses. We will record age if it is given.

** To avoid bias, an experienced preclinical researcher (TD) evaluates the validity of staining protocols for determining neuronal cell death/injury – blinded to the outcomes. Validity will be assessed based on the choice of staining or stainings, and the choice of structure or structures evaluated – including protocols with appropriate staining for neuronal cell death/injury and appropriate structure, excluding protocols evaluating non-specific cell distress or glial activity. If TD is unsure about the validity of a staining he will consult TC, and they will decide together if the staining is appropriate.

**Exclusion**
– Temperature reduction is induced pharmacologically.
– Cooling/heating was used only to prevent spontaneous temperature change without a corresponding control group.
– Data could not be used for meta-analysis, for example no information on group size or variance.
– Studies using animals treated with therapies adjuvant to the temperature reduction will be excluded, but where it is possible to extract data for a temperature reduction group without adjuvant therapy and a control group without adjuvant therapy these will be included.
– The study researches the benefits of temperature reduction to treat newborns.
– Studies exploring either Deep Hypothermic Circulatory Arrest or Cardiopulmonary Bypass together with temperature reduction are excluded if they do not specifically explore these as treatments of cardiac arrest.

• **Search strategy**

Embase & PubMed will be searched with no restriction to time of publication or language.

Rationale for search strategy: We consider it very unlikely that a study fulfilling our inclusion criteria will not contain 1) a synonym to cardiac arrest or ischemia (broad search) AND 2) a synonym to hypothermia or temperature reduction (in title or abstract) AND 3) a synonym to brain or its structures (broad search).

**Search strategy for PubMed:**

(cardiac arrest OR circulatory arrest OR ischemia OR ischaemia OR hypoxia OR anoxia OR infarct OR infarction OR asystole OR resuscitation) **AND**

(hypothermia>Title/Abstract) OR hyperthermia>Title/Abstract) OR normothermia>Title/Abstract) OR temperature>Title/Abstract) OR thermoregulatory>Title/Abstract) OR thermoregulation>Title/Abstract) OR chill therapy>Title/Abstract) OR cooling>Title/Abstract) OR cryotherapy>Title/Abstract)) **AND**

(brain OR hippocampus OR thalamus OR striatum OR cortex OR neuroprotecti* OR cerebral OR cerebrum OR neuron OR neuronal)

Entire string is pasted in the search field.

**Search strategy for Embase:**

('cardiac arrest' OR 'circulatory arrest' OR ischemia OR ischaemia OR hypoxia OR anoxia OR infarct OR infarction OR asystole OR resuscitation) **AND** ('hypothermia':ab,ti OR 'hyperthermia':ab,ti OR 'normothermia':ab,ti OR 'temperature':ab,ti OR
'thermoregulatory':ab,ti OR 'thermoregulation':ab,ti OR 'chill therapy':ab,ti OR 'cooling':ab,ti OR 'cryotherapy':ab,ti) AND (brain OR hippocampus OR thalamus OR striatum OR cortex OR neuroprotecti* OR cerebral OR cerebrum OR neuron OR neuronal)

Entire string is pasted in search field with the setting "search as broadly as possible".

The search strategy was discussed with a librarian. Multiple variations of both synonyms and block constructions were tested before arriving at the final search strategy.
**Data collection processes**

Articles from Embase and PubMed-search will be entered into EndNote X7 software. The EndNote library will be exported to CAMARADES software, which will be used for reviewing abstracts and full-text articles. Duplicate articles will be removed both automatically (using EndNote function “Remove duplicates”, author-year-title-reference type) and manually since Embase and PubMed reference the same article differently sometimes.

Independent reviewers will screen for relevant articles looking at title and abstract – each abstract will be screened by two independent reviewers. HO, GT and HW are primarily responsible for the screening. Their lists will be merged and included articles will be reviewed in full-text. Full-text review will be performed independently by HO, GT and HW. Articles not meeting all inclusion criteria or fulfilling one or more exclusion criteria will be excluded. Reason for exclusion will be noted. Disagreements between the three reviewers regarding inclusion/exclusion of full-text articles will be solved by additional review by three extra reviewers (TD, NN, JR) and, if necessary, a consensus discussion. A flow diagram of included and excluded studies at each stage will be presented in accordance with PRISMA-guidelines (17). A hand-search of the reference lists in the included articles will be performed, looking for articles investigating temperature reduction after global ischemia. As a final complement to the database search, relevant reviews on the topic will be searched for original articles. The gathering of reviews is arbitrary, not systematic, and relies on the years of experience of senior investigators. The database search will be updated towards the end of the project to allow for newly published articles to be included.

Included articles will be assessed qualitatively, quantitatively and with a modified STAIR-criteria. Data extraction will be performed independently by HO and GT. Disagreements will be solved by discussion, if not, then with the help of TD. Aside from publication ID, author surname and initials, journal, year of publication – qualitative, quantitative and modified STAIR-criteria data will be extracted in a predefined data extraction sheet (see below). Regarding quantitative data, several stratifications will be recorded (see below). If data is only presented in graphical form, a computerized ruler software will be used to
measure graphs (FlexRuler version 2.3, DropFrame). If data is incomplete we will contact authors.
• **Data analysis plan**

**Qualitative**

Individual studies will be checked using eight criteria outlined below. We arrived at this list by using CAMARADES quality checklist (18) and the checklist for assessing quality in studies researching the effects of hypothermia in focal ischemia models by van der Worp et al (19) and one additional criteria, number five.

1. Publication in a peer-reviewed journal
2. Randomization to treatment or control
3. Blinded induction of ischemia (i.e. concealment of treatment group allocation at time of induction of ischemia)
4. Blinded assessment of outcome
5. Statement of inclusion and exclusion of animals from the study
6. Sample size calculation
7. Statement of compliance with regulatory requirements
8. Statement regarding possible conflicts of interest

We will count the number of checklist items scored by each publication. If there is any doubt whether a study fulfills a certain criteria, no points will be given. Note that this is not an ordinal score, but rather a number of checklist items scored.

**Quantitative**

We expect great inter-study heterogeneity; therefore we will use a random effects model. We will use I-square to measure heterogeneity for the overall estimate of each outcome. We will set a lower limit of ten publications to perform the meta-analysis. Publication bias will be measured with funnel plot, Egger regression and trim and fill. CAMARADES software will be used for data analysis.

**Effect sizes**

Our primary outcome is neurobehavioural outcome; histological outcome and mortality are secondary. If more than one histological outcome or more than one behavioural outcome is reported from the same cohort of animals we will summarise these using fixed effects.
meta-analysis to provide a summary estimate of histological or behavioural outcome for that cohort. In each study the number of animals per group, mean outcome and standard error or standard deviation for both control and treatment group will be extracted. When a single control group serves multiple treatment groups, the control group will be divided by the number of treatment groups served (20). If outcome is measured serially (behavior, histology, mortality), the final measure will be extracted for meta-analysis. Ordinal data will be analyzed as continuous variables for the purpose of the meta-analysis.

For neurobehavioural and histological outcome it is unlikely that we can infer what a “normal” animal would score, therefore we will use standardized mean difference and not normalized mean difference (20). For mortality we will use odds ratio.

*Stratifications*

The following variables, and their effect on outcome, will be recorded from each study:

**Study design (15 items):**

Temperature reduction (7 items):

– Time to treatment (post ischemia)

– Method of inducing temperature reduction (intra- or extracorporeal or permissive)

– Depth of temperature reduction (temperature measured closest to brain)

– Duration of temperature reduction (start of temperature reduction defined as start of cooling and end of temperature reduction defined as end of cooling)

– Rate of rewarming (degrees/hour – start of rewarming defined as end of cooling and end of rewarming defined as the point when normothermia is reached or alternatively when rewarming is actively ended; temperature difference is defined as normothermia minus target temperature)

– Method of rewarming (intra- or extracorporeal or spontaneous)

– Method of controlling temperature in the control group (temperature management – normothermia or hyperthermia or no intervention*)

* See below

Ischemia (2 items):
– Model of global ischemia (selective arrest of cerebral circulation – e.g. 2-vessel occlusion, 4-vessel occlusion or cardiac arrest – e.g. by cardioplegic agents, exsanguination, asphyxia etc.)
– Duration of ischemia

Other (6 items):
– Time to outcome assessment (neurobehaviour, histology, mortality)
– Species
– Strain
– Sex
– Comorbid animals (diabetic, aged, hypertensive)
– Choice of anesthetic

Study quality (1 + 4 = 5 items, quality checklist items no. 2-3-4-5):
– Total study quality (according to quality checklist)
– The specific components of the quality checklist

Stratifications are sorted into two domains, study design and quality. Stratifications will be presented in a forest plot with point estimates and 99% (quality) and 99.67% (design) confidence intervals.

We will measure the significance of differences between n groups by partitioning heterogeneity and by using Chi-square test, n-1 df. To allow for multiple comparisons we will set a significance level at P<0.01 for the quality domain (n=5) and P<0.0033 for the design domain (n=15), the p-values were arrived at using Holm–Bonferroni correction. Since we use the statistically more conservative SMD we will partition heterogeneity for balance, instead of using the more conservative meta-regression (20). For continuous variables we will divide these into quartiles for partitioning of heterogeneity.

If a study uses more than one control (e.g. induced hypothermia vs. induced hyperthermia vs. induced normothermia) we will choose to extract the comparison/s according to a pre-defined list of priority:
1) induced hypothermia vs. induced normothermia
2) induced hypothermia vs. no intervention*
3) induced hypothermia vs. induced hyperthermia
4) permissive hypothermia vs. induced normothermia
5) permissive hypothermia vs. induced hyperthermia
* no intervention being either a) animals are allowed to spontaneously remain at normothermia after global ischemia or b) animals are allowed to spontaneously increase their temperature after global ischemia or c) animals are allowed to spontaneously decrease their temperature after global ischemia or d) unknown.

One experiment cannot contribute with two different statements about the same intervention (e.g. in the same study, induced 30°C vs induced 37°C would be the prioritized extraction above induced 30°C versus induced 40°C).

**Pre-specified sensitivity analysis**

We will perform sensitivity analysis if more than 150 outcomes are reported: where normalized mean difference calculation of effect sizes is feasible we will perform a sensitivity analysis using NMD, with meta-regression to explore the significance of differences between groups of studies.

We also anticipate issues suitable for sensitivity analysis to be identified during the review process – due to individual peculiarities of studies – which we cannot anticipate at the protocol stage.

**Modified STAIR evaluation**

We will use a modified STAIR-criteria to assess the scope of testing in the included studies, thus elucidating the preclinical evidence. This is a modified STAIR-criteria, similar to the one constructed by O’Collins et al. to evaluate neuroprotective drugs in models of focal ischemia (21) (22). We make a few departures from the original STAIR recommendations; we do not demand measures concerning quality control (randomization and blinding, since these are relating to quality within individual studies and this objective focuses on a cohort of studies). Some of the items in the study by O’Collins et al. are not applicable in our study, and we also added one item – Global ischemia model.

A clinically relevant temperature reduction will always have a specific combination of time to treatment, depth and duration. We will construct three matrixes (<2 hours time to treatment, between 2 and 6 hours, >6 hours), each with three durations and depths. Note that this allows for one study to contribute with more than one combination if it evaluates
different depths, durations or timings of temperature reduction. Other combinations of timing (pre- and intra-ischemic) are possible and will be recorded but not shown in the form of a matrix.

The modified STAIR-criteria consists of the following:

1. Laboratory setting – Combination tested in two or more laboratories*
2. Animal species – Combination tested in rodents and gyrencephalic species
3. Health of animals – Combination used in comorbid animals**
4. Sex of animals – Combination tested in male and female animals
5. Outcome measures – Combination evaluated with both histology and behaviour
6. Long-term effect – Combination has evaluated long-term outcome (either histology, mortality or behaviour, 4 weeks or more after ischemia)
7. Route of delivery – Combination tested with two or more methods of temperature reduction (e.g. intravascular cooling, extracorporeal cooling etc.)
8. Global ischemia model – Combination tested in two or more models of global ischemia where at least one model of global ischemia is accomplished by induced cardiac arrest

* By different investigators AND in separate laboratories
** Diabetic, aged, hypertensive

For each publication we will count the number of checklist items scored. If there is any doubt whether a combination fulfills a certain criteria, no points will be given. Note that this is not an ordinal score, but rather a number of checklist items scored.
Each box, i.e. a combination, can get at modified STAIR-score from zero to eight points. If target temperature is presented as XX degrees +/- Y degrees, XX will be used as depth of intervention. If duration of intervention is presented as XX min +/- Y min, XX will be used as duration and the same applies to time to treatment.

**Missing data**

**Qualitative**
If an article does not mention a qualitative feature in our data extraction sheet it will either be presumed not to have been performed (e.g. no mention of randomization will be interpreted as if it was not performed) or considered unknown (e.g. no mention of the sex of the animal used).

**Quantitative**
If an article does not mention a quantitative feature in our data extraction sheet it will be considered unknown and excluded from that analysis.
## Data extraction sheet

<table>
<thead>
<tr>
<th>Items</th>
<th>Study 1</th>
<th>Study 2</th>
<th>etc.</th>
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### Quality checklist

- Publication in a peer-reviewed journal
- Randomization to treatment or control (description)
- Blinded induction of ischemia (description)
- Blinded assessment of outcome (description)
- Statement of inclusion and exclusion of animals from the study (and number of animals included/excluded and for what reason)
- Sample size calculation
- Statement of compliance with regulatory requirements
- Statement regarding possible conflicts of interest

### Outcomes

- Number of animals per group (sham, control and intervention)
- Number of groups
- Mean outcome and standard deviation (histology, neurobehaviour and mortality)
- Method of neurobehavioural evaluation (description)
- Method of histological evaluation (description)

### Stratifications

- Timing of temperature management (pre-, intra-, post-ischemia)
- Time to treatment (post-ischemia)
- Method of inducing temperature reduction (intra- or extracorporeal or permissive)
- Depth of temperature reduction (temperature measured closest to brain)
- Duration of temperature reduction (start of temperature reduction defined as start of cooling and end of temperature reduction defined as end of cooling)
- Rate of rewarming (degrees/hour – start defined as end of cooling and end of rewarming defined as the point when normothermia is reached or alternatively when rewarming is actively ended; temperature difference is defined as normothermia minus target temperature)
- Method of rewarming (intra- or extracorporeal or spontaneous)
- Method of controlling temperature in the control group (temperature management – normothermia or hyperthermia or no intervention*)

Model of global ischemia (selective arrest of cerebral circulation – e.g. 2-vessel occlusion, 4-vessel occlusion or cardiac arrest – e.g. by
cardioplegic agents, exsanguination, asphyxia etc.)

Duration of ischemia

Time to outcome assessment (neurobehaviour, histology, mortality)

Species
Strain
Sex
Comorbidities (diabetic, aged, hypertensive)
Choice of anesthetic

_modified STAIR evaluation_
Laboratory setting (which laboratory/investigator was primarily responsible for the study)

**Intervention–Control set up (e.g. induced hypothermia vs. induced normothermia etc.)**
Comparisons of intervention–control in experiment

### Contribution of authors

HO – Hilmer Olai. Medical student, Lund University.
GT – Gustav Thornéus. Medical student, Lund University.
TD – Tomas Deierborg. Associate Professor, PhD, Lund University
NN – Niklas Nielsen
TC – Tobias Cronberg
HF – Hans Friberg
MM – Malcolm Macleod
JR – Jonathan Rhodes
HW – Hannah Watson

This protocol was written in the spring and summer of 2015 by HO and GT with the input from HF, NN, TC, TD, MM and JR.

The idea for the project was developed by TD, TC, NN and HF.

Screening of abstracts (independently) – primarily HO, GT and HW.
Full-text review (independently) – HO, GT, HW.
  disagreements regarding inclusion/exclusion – TD, NN, JR.
evaluation of histological protocols (blinded to outcome) – TD with support from TC
handsearch of reference lists in included articles and reviews (independently) – HO, GT, HW.

Data extraction (independently) – HO, GT.

Analyze data – Junior and senior investigators

Write report – Junior and senior investigators

• **Disclosures**
  None.

• **Funding**
  None.
References
