Protocol

Can Mild Induced Hypothermia reduce mortality in septic shock patients at the Intensive Care Unit? -A randomized, single-blinded, multicenter study The Cooling And Surviving Septic shock (CASS) study

Protocol version: 6.5

Date: 12 October 2011

Intensive Care Units from all over Denmark participate : This study is made by the project group around the Procalcitonin And Survival Study (PASS), a randomized controlled intervention study conducted in 1200 intensive care patients in 8 intensive care units in Denmark. A large and well-functioning organization was built up during the PASS study. The PASS group is invited to participate in the CASS study.

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” INVESTIGATOR PROTOCOL AGREEMENT PAGE

THIS AGREEMENT IS EQUIVALENT TO A “SIGNED PROTOCOL”

The CASS Study

Name and qualifications of investigator:

Name of Investigator: _____

Position held: _____

Clinical Centre: _____

I agree:

- to assume responsibility for the proper conduct of the CASS Study at this site.
- to conduct the trial in compliance with this protocol, any future amendments, and with any other study conduct procedures provided.
- not to implement any deviations from or changes to the protocol without agreement from the sponsor and prior review and written approval from the Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements).
- that I am thoroughly familiar with the appropriate use of the procedures regarding the use of Mild Induced Hypothermia and the adverse effects and contraindications for this treatment plus any other information manufactured by the CASS Coordinating centre.
- that I am aware of, and will comply with, ‘‘Good Clinical Practice’’ (ICH-GCP Guideline (CPMP/ICH/135/95, Directive 2001/20/EC)) and all applicable regulatory requirements.
- to ensure that all persons assisting me with the trial are adequately informed about the Procalcitonin test and interpretation and of their study-related duties and functions as described in the protocol.

_____ _____

Signature of investigator Date

One signed copy each to be held by the Investigator and CASS coordinating centre.

CONTENT

A randomized, open-label multicenter study to investigate whether Mild Induced Hypothermia can reduce mortality in Intensive Care Unit (ICU)-patients with septic shock
The Cooling And Surviving Septic shock (CASS) study

PROTOCOL SUMMARY

Inclusion:

Fulfillment of all of the following six criteria:

1 Aged ≥ 50 years of age.

2 Severe sepsis[1] /septic shock = →SIRS + suspected infection + hypotension →Mean Arterial Blood Pressure (MAP) <70 mmHg,

3 Admitted to the participating intensive care units (ICU)

4 Indication for intubation

5 Possibility of inclusion within 6 hours after septic shock/severe sepsis is diagnosed in the ICU. Patients admitted with septic shock/severe sepsis should be included within 6 hours after admission. If a patient is not included within this period, that patient cannot be included within the same hospitalization.

6 The patient must have an expected stay in the ICU of more than 24 hours. Anticipated death within 24 hours after admission to the ICU does not exclude participation; however no decision of reduction of treatment level must have been taken. During this time period, probability that the patient is discharged to a floor department must not be likely (<10% probability).

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Exclusion:

A subject will **NOT** be eligible for inclusion in this study if any of the following criteria apply:

1. Patients are pregnant or breast feeding
2. The findings of the initial screening, shows that the patient has a bleeding disorder and/or the patient has an uncontrollable bleeding and /or surgery within the last 24 hours
3. Persons who are detained under the Act on the use of coercion in psychiatry

Patients are included acutely and subsequent retrieval of either 1) informed consent from the patient or 2) vicarious informed consent. Please see instructions for this separately.

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[1] Because it is presumed to be an essential part of the intervention with early initiation, it would be irresponsible to wait any eventuel resuscitation of fluid therapy, as this would affect the time to active treatment.

Studies with incapacitated in acute situations, see Committee of legislation §20.
Patients with septic shock have a risk of dying at 50-80%. The acute lifesaving treatment of these patients upon the arrival to the ICU implies by default that the patient is acutely intubated (connected to mechanical ventilation) and thereby sedated. Therefore, because the patients are unconscious either due to the acute illness or due to the acute treatment, the septic shock patients are not able to consider participation in the CASS study.
To make clinical studies, with the goal of improving the treatment of these serious infections, it is necessary to include unconscious patients and patients with blurred consciousness. It is not possible to conduct this investigation with another (conscious) patient group and get the same result, since patients with infections without septic shock have a much lower mortality rate and because the pathogenesis of infection without circulatory collapse is very different. Subsequently there will be obtained a written informed consent from the patient himself or, if this is not possible, from close relatives and the patient's family doctor/medical officer.
Because septic shock is a condition which is acute life-threatening, with great organ damage for every hour, it is extremely important to initiate treatment rapidly (within minutes to few hours). Conversely, bacterial infection is a reversible condition and, if the patient survives, the patient's health is expected improved completely, so that the patient in most cases would completely recover and resume (pastime) work and family functions.
The probability of *a priori* to survive with 1) the recommended diagnostic and treatment with the currently available means to identify infections (standard of care) and on the other side by 2) the recommended diagnostic and treatment with the currently available resources to find infections (standard of care) **and cooling to 33^0 C for 24 hours after the onset of septic shock, must be equal**. This means if during the study evidence arises which can positively determine that one treatment has either a better or worse outcome for patients with septic shock, the study is stopped.

Randomization:

560 patients in two arms (1:1), n = 280 per arm:

> **Arm 1**: Recommended diagnostics and treatment of infections in the intensive care unit (standard of care)
> **Arm 2**: Recommended diagnostics and treatment of infections in the intensive care unit (standard of care) **PLUS** cooling to maximum 34^0 C aimed within 2 hours after enrollment and maintained for 24 hours. The patient is subsequently heated and kept normothermic (body temperature of 36 ^{0}C - 38 ^{0}C) for 72 hours from start of randomization (day 0), then no further intervention regarding temperature control.

Primary Study Objective: To investigate whether cooling to 33 °C for 24 hours following diagnosis with septic shock is found to reduce mortality in intensive care patients.
Registration Days in the study: ICU admission day, ongoing daily routine recording of examinations and blood tests, discharge or death, day 30, 60, 90, 120, 180 after discharge.
Data collection: Data collection will be simple and done "real time" via Fax.
Biobank: A research biobank is created during this study.

1 STUDY BACKGROUND AND RATIONALE

1.1 Background

1.1.1 *Sepsis and mortality at the ICU*

Mortality in septic shock (circulatory collapse associated with serious bacterial infection), remains exceedingly high, in some cases around 50% (1, 2).
Within the last 10 years the understanding of the serious changes in the circulation that occurs in septic shock has increased significantly: Blood Circulation in the smallest blood vessels (capillaries) degrades due to blockage by blood cells - a process initiated by substances from the cells of the immune system via activation of coagulation (3-5). In this process the level of free oxygen and nitrogen radicals plays a central role (6).
The normal function of the smallest blood vessels is to transport oxygen, nutrients and drugs to organs and tissues, and lead waste products away. While the offer of oxygen and nutrients to the organs decreases, the consumption of oxygen and nutrients increases due to fever and immune reactions.
When this "micro circulation" collapses, the organs and tissues suffer, and various forms of cell death in the organs begins including “programmed cell death” (“apoptosis”) (7, 8). This leads to organ damage, for example brain damage or kidney damage and ultimately to multiple organ dysfunction which is the direct cause of the patient dies.

Mild induced hypothermia (cooling to 32 ^{0}C–34 ^{0}C) affects at least 4 core areas in the pathophysiology of septic shock: 1) inhibition of inflammation and apoptosis ("programmed cell death”), 2) antithrombotic, 3) decreases the metabolism and 4) inhibits bacterial growth and production of toxins.

1.1.2 *The Effect of Mild Induced Hypothermia on inflammation and apoptosis (”programmed cell death”)*

In has been observed in animal studies that cooling to 33 °C (Mild induced hypothermia, MIH) exhibit an anti-inflammatory effect, especially through decreased release of TNF-α, IL1-beta, IL6, ICAM-1, GRO/CINC-1 and myeloperoxidasis (9-11) and by decreased production of free radicals through the nitrogen iNOS system (12-14). These inflammatory mediators that stimulate apoptosis are thereby inhibited by MIH (15). Furthermore, local extern cooling inhibits the TNF-α induced leukocyte adhesion and capillary leakage in microcirculation (15). Studies on humane umbilical endothelial cells shows that cooling to 33 °C inhibits the activity of caspases and BAX, a group of apoptosis promoting proteins, while the activity of the apoptosis inhibiting protein Bcl-2 increases (16).

1.1.3 The effect of Mild Induced Hypothermia on coagulation

Basically, the effect of anticoagulation is desired cf. the above mentioned mechanism which are responsible for the breakdown of the microcirculation, however any anticoagulation has a risk of side effects.

A mild anticoagulant effect has been the subject of several large randomized trials among severely infected patients. All these studies have used drugs (which are dependent on a functioning microcirculation) and have not shown convincing efficacy (17-19), perhaps exactly because drugs are dependent on a functional microcirculation.

Hypothermia has anticoagulant properties and at a temperature below 34 °C tromboxane B2 is inhibited, thereby reducing platelet aggregation and the rigidity of erythrocytes which in septic shock contributes to breakdown of the capillary system. At temperatures below 32 °C the synthesis of coagulation factors is reduces which further inhibits the ability of blood to clot (20-22).

Thus, MIH will inhibit platelet aggregation but has no immediate effect on clotting factors. This is supported by clinical studies in which bleeding complications during MIH are rarely observed (23). Other studies have shown that MIH (to 34 °C) results in reduced occlusion time in vessels containing blood clots, whereas cooling to lower temperature exhibits the opposite trend (24).

1.1.4 Effect of Mild Induced Hypothermia on the metabolism

Metabolism decreases 5-9% per 1 °C that the body temperature is lowered thereby reducing the need for energy, oxygen and production of carbon dioxide (25, 26). This means that each cell can survive longer in patients with compromised microcirculation and thus a lower supply of nutrients and oxygen (eg. septic shock).

1.1.5 Temperature, bacterial growth and production and toxins

In a study of bacterial growth and its dependence on temperature a strong increase in growth velocity was found as a function of temperature.

The lowest growth rate for E. coli was found at 50 ^{0}C and an increase in growth rate was observed up to about 40 ^{0}C, after which the rate decreased.

At 33 ^{0}C it was found that the square root of the growth rate (per hour) was approx. 1.3. At 38 ^{0}C the square root of the growth rate was about 1.55. Consequently the growth rate at 33 ^{0}C is $(1,3)^2$ = 1.69 x numbers of bacteria per hour at the starting point and at 38 ^{0}C the growth rate is $(1.55)^2$ = 2.40 x numbers of bacteria per hour at the starting point.

The results were studied for 10 different strains of E. coli to test if this only applied to one E. coli strain. However all strains showed the same dependence on temperature in growth rate. Similar results were shown for Bacillus cereus. Listeria monocytogenes also demonstrated temperature

dependence, although it was somewhat less (27).

E.coli is the most commonly cultured microorganism from blood cultures in most microbiology departments, and hence on the clinical microbiology department at Hvidovre Hospital (unpublished data).
It is well documented that the median time to correct treatment of septic shock is (or at least recently has been) about 6 hours in Western university hospitals (28). Furthermore the time, before the antibiotics has reached the infected area and is working on the infection must be added.
The difference in bacterial growth during the interval for proper treatment can thus be approximated:
33^0C: At T = 6 hours after the starting point are: $(1.69)^6$ = 23 times more bacteria.
38^0C: At T = 6 hours after the starting point are: $(2.40)^6$ = 191 times more bacteria
By lowering the temperature from 38 ^{0}C to 33 ^{0}C in a cell culture there are 10 times fewer bacteria after 6 hours compared to keeping the temperature at 38^0C
In a study of E. coli and Pseudomonas aeruginosa characteristics during different incubation temperatures it was found that in the temperature range 20 ^{0}C – 30 ^{0}C there was almost no production of endotoxin / lipopolysaccharide. Subsequently, production increased sharply up to 48 ^{0}C (29).
These facts are consistent with general knowledge regarding chemical processes and enzymatically catalyzed processes that speed up at higher temperatures until the enzyme/protein, as a “build in break” begins to denature and the biological function is lost.
Thus there is evidence that both bacterial growth and bacterial endotoxin production is highly dependent on temperature, also in the range between 33 ^{0}C and 38 ^{0}C.

1.1.6 Hypothermia and animal studies

Induced Hypothermia on rats injected with endotoxins

Endotoxins/ lipopolysaccharides are part of the gram-negative bacterial cell wall. Endotoxins are the cause of many of the pathophysiological changes that occurs in bacterial infections. In an animal study, 24 rats given 10 mg / kg endotoxin injected into the abdominal cavity (peritoneum) were randomized to either hypothermia (n = 12) or normothermia (n = 12). Survival was 2/10 rats (20%) in the normothermia group (2 died during the surgical procedure) and 10/12 (83%) in the hypothermia group, p = 0.0083 (30).
The experiment was repeated with a new group of rats and this time the dose of endotoxin was increased to 20 mg/kg. This time as well the survival rate was increased in the group receiving hypothermia from 1 /10 to 5/10.
In a third study the application of hypothermia after surgery (instillation of endotoxin) was postponed for 1 hour. This study was performed to simulate a more common situation in human

infection, as the time from the patient arrives at the hospital/the appropriate treatment/intensive care unit is often minimum 1 hour. Again, it appeared that mortality was reduced significantly by hypothermia, as the group receiving hypothermia treatment had a survival rate at 5/10 rats compared to 1/10 survivors in the group receiving normothermia.
The 62 rats were randomly assigned to either hypothermia or normothermia, the overall statistic is made up below:

	Non-survivors	Survivors
Hypothermia	12	20
Normothermia	26	4

Table 1. Data on mortality, Huet et al. (30)

Relative risk of death = 0.43. [95% CI: 0.27 to 0.69], p <0.0001. (Total statistic calculated by the CASS study group, as overall statistics were not shown in the publication, Fisher exact test and Chi-square for equal proportions, same result).

L'Hers group developed an animal model of sepsis caused by cecum ligation and perforation of the intestine. The animals were randomized into hypo-, normo-or hyperthermia. The results showed a significantly increased survival in animals receiving hypothermia compared with normo-and hyperthermia. Animals in the hyperthermia group had the lowest survival rate (31).

1.1.7 Cooling and human studies

Cooling patients after cardiac arrest

273 patients with cardiac arrest due to diagnosed ventricular fibrillation (VF) were randomized to either the usual follow-up treatment after cardiac arrest or this plus mild induced hypothermia with target temperatures of 32^0 C - 34^0 C for 24 hours after resuscitation. Mortality was 39% in the hypothermia group vs. 55% in the normothermia group, the Hazard Ratio for the normothemia group: 1.40, 95% CI [1.08 to 1.81]. Mortality after 6 months were also different, 41% had died in the hypothermia group and 55% in the normothermia group, Hazard Ratio: 0.74, 95% CI [0.58 to 0.95] for the hypothermia group. Complication rates were also comparable in all other measured points HACA study (23).

Cooling of other non-infected patients

In a group of 208 children with neonatal hypoxic /ischemic encephalopathy that received mild induced hypothermia, the incidence of death or moderate to severe brain damage was reduced from 62% to 44%, hazard ratio 0.72 95% Confidence interval [0.54 to 0.95], p = 0.01 (32).

Cooling of infected adult patients

Induced hypothermia

To our knowledge there has only been one study that systematically, and not only accidentally, used treatment with mild induced hypothermia in infected adults. In this study, 19 adult patients with "Adult respiratory distress syndrome" and sepsis where selected consecutively to normotermia (n = 10) and hypothermia (n = 9) (24).

The main result was that a significantly higher proportion of the supplied oxygen was absorbed by the patient, expressed by an increased PaO_2/ PAO_2 ratio and an enhanced O_2 extraction in the hypothermia group compared with the normothermia group. Furthermore the heart rate fell from median 117/min to 96 per minute.

Such a decrease in heart rate would mean that the heart's oxygen consumption drops significantly. In the aforementioned study, the total oxygen consumption remained constant in the hypothermia group, which means that the oxygen that is "spared" in the heart must have been consumed by other organs. This despite the decrease in metabolism because of the reduced temperature. Therefore the study indicates that even with reduced metabolism, there is an increase in oxygen demand/offer in the organs of patients receiving mild induced hypothermia.

The study has some shortcomings, including the very low sample size and lack of observation/ reporting of a range of circuit parameters. Despite this, a number of useful information can be extracted, since it serves as a cohort study with a control group *and* as a crossover study, since all the physiological parameters are measured during an observation period before cooling of the group receiving Mild Induced Hypothermia. Before cooling, there were no physiological parameters which differ between the hypo-and the normothermia group, like a comparison between the hypothermia group (before cooling) and the hypothermia group (during cooling) shows the same differences, that the ones that exist between the hypothermia group and the normothermia group. Mortality: In normothermia group 10/10 patients died = 100% mortality and for the group receiving hypothermia 6 / 9 = 66.6% died, p = 0.08 [the authors state a significant result, but the CASS project group has corrected this to 0.08 see results from both the Chi-square test and Fischer test].

Induced normothermia

One of the major pathophysiological mechanisms in the development of septic shock is the inability to maintain blood pressure and thus organ perfusion.

Patients with septic shock are routinely treated with "inotropic" substances such as dopamine, dobutamine, noradrenaline and adrenaline.

A recent multicentre study included febrile patients with septic shock (body temperature of 38,3 ^{0}C and the need for inotropic agents). The patients were randomized into 2 groups; one group was cooled to normothermia (36,5 ^{0}C – 37 ^{0}C) for 48 hours. The study showed that the group cooled to normothermia had a reduced need for inotropic agents than the group of patients with fever (33).

Thus, fever had, in this study, a deleterious effect, which is also found in other studies including Atwood and Kass' animal models with induced fever (34). This adverse effect may be explained by the fact that fever stimulates apoptosis-inducing proteins (35).

1.1.8 Brief review of relevant background literature - Table 2

Keywords at Medline/Pubmed: [hypothermia AND sepsis] of all search terms (TI, etc.) and without "limits ".

This broad search produces 509 articles (December 2010). The title of all these have been read by members of the CASS project group. If the title suggested that the article was dealing with hypothermia and infection the abstract was read. If abstract 1) referred to hypothermia (accidental, spontaneous or induced) and bacterial infection and was NOT a case story, the article was obtained. In this way, 22 relevant articles were obtained. These are introduced in the table below.

Author	Method	Sample size N	Main Results	Criticism of the method, statistical or design
Fujimoto (10) ICAM-1 = intercellular adhesion molecule	-Rats, acute peritonitis (AP) and endotoxins. -4 groups: 1) Sham (-cerulein, -LPS,normotermia), 2) AP +LPS (control), 3) AP + LPS + early hypotermia 32 ^{0}C, 4) AP + LPS + late hypotermia 32 ^{0}C	N=36 9+9+9+9	-IL-6 in both hypothemia groups. Lower the in the control group -IL-10 higher v. early hypothermia then in all the other three groups. -s-ICAM-1 lower in cooling then among controls plus lower at early then late cooling.	Endotoxaemia/not bacteraemia. Does not allow extrapolation to clinical situations with bactaeremia + endotoxaemia. Not randomized and not blinded.
Kanakura (36)	Rats ->Two protocols: 1.Moderate hypothermia 30-32 ^{0}C, 2. Mild hypotermia 33-35 ^{0}C. 4 groups in each protocol: A) E.coli LPS + normothermia, B) As A + propofol, C) E.coli LPS + hypothermia, D) As C + propofol.	N=88	Mortality: Normothermia groups: 40-70% Hypothermia groups 0-10% No additive effect by simultaneous administration of propofol.	Endotoxaemia/not bacteremia. Hemodynamic monitoring limited. Group A has lower blood pressure and lower pH than others. Monitoring- only 6 hours.
Hofstetter (37)	Rats a) Sham, b) LPS alone, c) LPS-hypothermia, d) LPS-sevoflouran, e) LPS-sevoflouran-hypothermia Comparison of the anti-inflammatory effect of Sevoflouran and Mild Induced Hypothermia	N = 30 n = 6 per group 5 groups:	Hypothermia alone and together with sevoflouran lowers TNF-alpha with 46-58%, but not IL-1 beta. IL10 is significantly higher significant in hypothermia, but not in sevoflouran	Endotoxemia/ not bacteremia. The results are based alone on interleukins and AM nitrite. Survival not stated.
Lindenblatt (24)	Rats. Experimental design. 10(Nacl37^{0}C)+ 8 (LPS 37^{0}C)+ 8 (LPS 34^{0}C)+ 8	N=34 (17 animals)	Systemic hypothermia 34^{0}C →reduction in time of occlusion	Endotoxemia /not

	(LPS 31^{0}C) Study to determine if hypothermia affects the speed of micro thrombosis by LPS intraperitoneally.			bacteremia.
Kuboki (38)	Mice. Experimental design Effects of hypothermia on NF-Kappa-B activation and liver injury. 90 minutes time of ischemia followed by 8 hours reperfusion period.	N = 24 4 groups á n=6	Liver damage expressed by histopathology markedly reduced by hypothermia, linear relationship (lower tp → less damage). Inflammation (TNF-alpha and neutrophile recruitment) was lowest in hypothermia groups	An animal experimental study focusing on ischemic reperfusion damage in liver cells.
L´Her (31)	Prospective, randomized animal study (rats). Sample size based on power calculation. Sepsis by cecum ligation and perforation and subsequent randomization to hypo-, normo-or hyperthermia.	N=18 3 Sub groups n=6: a) 42 ^{0}C b) 37^{0}C c) 32^{0}C	Increased survival among animals receiving hypothermia in experimental sepsis. Decreased survival of heated animals.	-NO AB - Hemodynamic not monitored. -42^{0}C unphysiological.
De Pont (39)	Editorial following L´Her´s article (6)		-Normothermia not evidence based therapeutic goal for sepsis -Argues randomized human study with cooling	Evidence grade.
Su (40)	Sheep, Sepsis-study. -Laparotomy and cecal perforation - Allocation to the hypothermia group if spontaneously fall in Tp: TP<36^{0}C. -Animals randomized to 3 groups: T>39, 37,5<T<38,5, 36<T<37.	N=24 (n?)	Longer survival at TP>39 PaO2/FiO2 higher and se-lactat lower at TP>39 the in other groups	Mixer spontaneous hypothermia and induced hypothermia. Spontaneous hypothermia is well documented poorly prognostic.
Torossian (41)	Randomized. Rats. -Peritonitis with the inoculation of human faeces in the peritoneum. -3 groups: 1) Normotermi (n = 42), 2) Mild hypothermia (n = 42) and 3) non-septic mild hypothermia (n = 12). -Primary endpoint: death by 120h	N=96	Survival at 120 hours: -Hypothermia: 50% -Normothermia: 75% -Controls: 100% Hypothermia affects leukocyte recruitment and affect the cytokine balance compatible with immune pareses	-Hypothermia only 1 hour -After 1 hour, heating to 38^{0}C using infrared heat lamp→Unresolved if the hypothermia group die due to the heating to 38^{0}C with the infrared lamp.
Bota (42)	Human clinical observational study mixed ICU patients Interventions: none Fever> 38.3 Hypothermia <36 Three groups: 1) Spontaneous hypothermia (at Any Time) n = 45 2) Normotermia (at all times) 3) fevers (at Any Time) n = 139	N=493 Patients divided into 3 groups	Fever/hypotermia Associated with: >infection >SOFA > LOS >mortality The observed among normothermia	Only spontaneous hypothermia examined, this has been documented to be associated with poor prognosis.
Scumpia (13)	Experimental design. Rats Endotoxaemia in 2 groups a) Cooling 18-24 ^{0}C and b) normotermia 36-38 ^{0}C. Plus c) Placebo (sham)-operated control group Monitored in 150 min. and euthanized	N=15 Hypotermia+LPS n=6 Normotermia+LPS n=6 Sham=3	Hypothermia induces myocardial expression of inflammatory cytokines and inhibit the expression of pro-inflammatory cytokines. iNOS messenger RNA and- protein mgd. Are reduced as is myocardial myeloperoxidasis	The study is assessed due to the extreme degree of hypoxia and the short observation time, not relevant in clinical settings.

Fairchild (43)	Cell culture (THP-1 human prononcyte)	?	Delayed onset TNF-a, ILb1-mRNA and cytokine. Once initiated expression is augmented and prolonged.	Cell culture. Important findings that onset is delayed
Taniguchi (11)	Rats, pentobarbital anesthesia. LPS (E.coli). 30-31, 34-35, normothermia. Samples after 2,5,6 t.	36 (3x12)	Hypothermia: decreased mortality. Impaired inflammatory response and lower NOx in tissues.	Short time period. Maximum 6 hours.
Sarcia (9)	Rats anesthesia. Halothane, ketamine, xylazine. LPS (E. coli). Lung tissue harvested after 150 min	2x6	Hypothermia→ Intra pulmonary reduction of IL1beta, IL6, GRO/CINC-1, myeloperoxidase. Reduced histological inflammatory response (edema, Neutrophil acc).	In vitro study. Short time period (150 min)
Silveira (44)	Neonates hospitalized with spontaneous hypothermia	320 (106 spontaneous hypothermia)	spontaneous hypothermia at admission → 3-fold increase in mortality	Spontaneous hypothermia. The model does not consider other facts.
Scumpia (12)	Rats anesthesia. Halothane, ketamine, xylazine. LPS (E. coli). After 150 min measuring of NO.	3 sham,6 18-24 ^{0}C, 6 36-38 ^{0}C	Hypothermia protects against intrapulm. NO over production and NO-mediated lung injury by inhibiting the transcription of Inos, CAT-1 and CAT-2	
Marik (45)	Septic shock pt. (Pt. I NORASEPTII study – placebp limb) Tp., IL6, TNFa, mm. Spontaneous hypothermia	930 pt. (195 hypothermia <35,6 v at hospitalization)	Lower survival in spontaneous hypothermia. Hypothermia may be explained by lower production of proinflammatory cytokines	Spontaneous hypothermia Hypothermia is <35.6 Retrospective study
Arons (46)	Sepsis patients randomly assigned to ibuprofen or placebo iv Concerning hypothermia: looking at spontaneous hypothermia 44 spontaneously hypothermia <35.5 ^{0}C 409 fever> 38.3 ^{0}C 2 Normothermia (exclude.)	455 patients with sepsis	- 10% incidence for hypothermia in sepsis. - Mortality x2 in hypothermia sepsis sml. M. febrile - Hypothermia sepsis -> enhanced response of TNF-a, IL-6, TxB2, prostacykline. -Possible reduction in mortality by ibuprofen	Spontaneous hypothermia in sepsis. Immunmodulation with NSAID.
Villar (47)	Prospective controlled study. ARDS + Sepsis Randomization to standard treatment or hypothermia (32-35 degrees) + standard treatment.	19 pt with ARDS + sepsis 9 hypothr. + standard treatment and 10 standard treatment	Hypothermia→ Reduces mortality Reduces P(A-a).	Small sample size. Pilot study
Membré (27)	Study on the growth rate of Listeria, Salmonella, E.coli and Clostridia by tp. Between 2 and 48 degrees. Objective: To identify variability and recognize this in a mathematical model for bacterial growth In foods.		For the tested bacteria: E. coli, L. monocytogenes, B. cereus was growth rate lower at 32 ^{0}C then at 37 ^{0}C or higher. Discussed above in the background section of the text.	Mathematical model development a view of predicting bacterial growth in foods at different tp.

Weber-Frick (29)	Growth rate of Pseudomonas and E. coli in growth medium. It is tested for the amount of LPS produced by different tp.		Lowest LPS production at 20-30 degrees	In vitro study of a view on how the quantity of bacterial growth in foods
Huet (30)	Rats, anesthesia + intubation. Series 1: Randomization to hypothermia 32 degrees and normothermia. Intraperitoneal LPS 20 mg / kg. 2 hours later blood samples extubation. 7-day mortality. Series 2: As serie1 but hypothermia started 1 hour after LPS administration 10 mg / kg.	64 male Sprague-dawley rats (4 x 16)	Hypothermia→ improved survival	Good animal study.

Table 2, background literature. Studies in **bold** are considered central (Key studies).

Key studies are selected based on the following criteria: 1) do not involve spontaneous hypothermia, 2) do not use hypothermia below 31^0C, 3) do not reheat animals within 4 hours. In addition, laboratory studies of bacterial growth rates and toxin production are included as well as a human pilot study is included despite a number of methodological weaknesses, since this is the only human study to date.

1.2 Rationale – summery

(References above)

Septic shock patients still have approx. 50% risk of death.

There is evidence in the currently available literature that mild induced hypothermia inhibits apoptosis both directly and by inhibition of inflammatory mediators, has an anti-thrombotic effect especially by inhibiting platelet function, reduces metabolism by 5-9% per degree Celsius of lowering (corresponding to 35%-50% total metabolism lowering in the proposed range), and inhibits bacterial growth and production of toxins.

Furthermore, it is well documented that hypothermia has considerable effect in other conditions where there is great risk of tissue damage and organ failure, e.g. cardiac arrest and neonatal hypoxic /ischemic encephalopathy. Several experimental animal models with induced sepsis have shown improved survival when treated with induced hypothermia. Rats with endotoxins instilled into the abdominal cavity, treated with mild induced hypothermia have better survival than the control group who did not receive hypothermia [Table 2]. Similar results are demonstrated in a couple of human studies. Patients with septic shock cooled to normothermia, had a reduced need for inotropic substances compares with febrile patients. Another study included 19 patients with Adult Respiratory Distress Syndrome and sepsis. In this study the group treated with hypothermia had a significantly improved effect on heart and lung parameters and a trend towards better survival compared to group that did not receive hypothermia.

1.3 Hypothermia: Procedure to induce hypothermia

Procedures for treatment with hypothermia, therapeutic mild induced hypothermia, is conducted by the same procedure as after cardiac arrest. The procedure for this is attached, see appendix 3.

1.4 Rationale for a 24-hour cooling period

The above documentation is based on the patophysiological changes occurring within the first 24 hours after the initial stimulus. Inhibition of bacterial growth is most important within the first day, since one must assume that antibiotic treatment has taken over in terms of inactivation of bacterial growth and stop production of toxins. Studies regarding cardiac arrest and mild induced hypothermia have formed evidence based on a 24-hour cooling period.

2 STUDY OBJECTIVE AND ENDPOINTS

2.1 Primary Objective

The investigate whether cooling to 33^0 C in 24 hours can reduce 30-days ”all-cause” mortality among patients in the Intensive Care Unit

2.2 Secondary Objectives

1. To determine the mortality of ICU patients receiving MIH for 24 hours compared with ICU patients that do not receive MIH, at different time points after day of admission
2. To determine the effect of MIH on the duration of septic shock
3. To determine the effect of MIH on the duration of mechanical ventilation
4. To determine the effect of MIH on the cerebral status
5. To determine the effect of MIH on the renal function
6. To determine the effect of MIH on the duration and degree of liver involvement
7. To determine the effect of MIH on the coagulation
8. To determine the effect of MIH on the progress of infection

2.3 Study Endpoint(s)

2.3.1 Primary Endpoint:

30 day all-cause mortality after inclusion in the CASS study

2.3.2 Secondary Endpoints:

1. Duration of cardiac/septic shock: A) Delta Mean Arterial Pressure (MAP).
   Analysis: MAP (day 4, a.m. 4:00 to 9:00) – MAP (day 1).
   B1) Inotropic score day 1, day 2, day 3, day 4 (time 0, 24, 48 and 72 hours); = (dopamine doses x 1) + (dobutamine doses x 1) + (adrenaline doses x 100) + (noradrenaline

	doses x 100) + (phenylephrine doses x 100), doses given in µg/kg/min. B2) Inotropic score day 1+day 2 + day 3 + day 4 (time 0, 24, 48 and 72 hours) (accumulated need for inotropic) C) Achieved discontinuation of vasopressor/inotropic on day 2, day 3, day 4 (a.m. 4:00 to 9:00). SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 50%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,25 (requires 480 patients)
2. Respiratory failure:	A) Delta PaO2/FiO2 ratio. Analysis: PaO2/FiO2 ratio (day 3 and day 4, a.m. 4:00 to 9:00) - PaO2/FiO2 ratio (day 1) B) Achieved discontinuation of mechanical ventilation on day 3, day 4 (a.m. 4:00 to 9:00). SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 40%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,29 (requires 560 patients)
3. Renal failure:	A) Acute renal failure cf. the RIFLE criteria. Analysis: 1) Increase in serum-Creatinine at x 1.5 at any time from day 1 = time of randomization before induction of hypothermia and day 4 (a.m. 4:00 to 9:00) OR decline in eGFR of> 25% (both providing endpoint). Analysis 2) Increase in serum-Creatinine at x 2.0 at any time from day 1 = time of randomization before induction of hypothermia and day 4 (a.m. 4:00 to 9:00) OR decline in eGFR of> 50% (both providing endpoint). Analysis 3) Increase in serum-Creatinine at x 3.0 at any

	time from day 1 = time of randomization before induction of hypothermia and day 4 (a.m. 4:00 to 9:00) OR decline in eGFR of> 75% OR serum-Creatinine ≥300 μmol/L (all three providing endpoints). SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 30%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,38 (requires 560 patients)
Cerebral:	A) Delta Richmond Agitation Sedation Scale (RASS) Analysis: RASS (day 4, (a.m. 4:00 to 9:00) – RASS (day 1) B) NEGATIVE: Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) 24, 48 and 72 hours after Awakening. SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 60%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,19 (requires 560 patients)
5.Hepatic:	A) Delta Bilirubin. Analysis: Bilirubin (day 4, (a.m. 4:00 to 9:00) - Bilirubin (day 1) B) Achieved Bilirubin >=21 mM at 72 hours (day 4) SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 20%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,5 (requires 560 patients)
6.Coagulation:	A1) Delta platelets.

	Analysis: platelets (day 4, a.m. 4:00 to 9:00) – platelets (day 1) A2) Delta platelets/binary: Achieved decrease in platelets >25% day 1 → day 4. B) Delta INR. Analysis: INR (day 4, a.m. 4:00 to 9:00) – INR (day 1) C) Delta Factor II, VII, IX (”PP”). ”PP” (day 4, a.m. 4:00 to 9:00) – ”PP” (day 1). D) Delta APTT. APTT (day 4, a.m. 4:00 to 9:00) – APTT (day 1) E) Total consumption of SAG-M the first 10 days F) Occurrence of severe bleeding (1) CT confirmed ICH, 2) fresh upper or lower gastrointestinal bleeding 3) other bleeding with need for surgery). G) Trombelastrography (TEG) or tromboelastrometry (ROTEM) is executed in the departments who have the opportunity. (Time R/CT (s)) Angel, MCF/MA. Day-to-day variability (unilateral variation analysis: ANOVA) Lysis30 min (lysis 30 min after maximum amplitude) SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 35%. <u>Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,33</u> (requires 560 patients)
7.Infection:	A) Quantitatively: Delta C-reactive protein (CRP). Analysis: CRP (day 4, a.m. 4:00 to 9:00) – CRP (dag 1) B) Binary: Achieved decrease in CRP >30% from day 1 → day 4. SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 35%. <u>Limit for reliable detection of relative difference (both</u>

	ways) to the occurrence of endpoint in the hypothermia group: 1,23 (requires 560 patients)
8. Days without organ failure up to day 30	Organ failure is defined by: the need for mechanical ventilation, need for inotropic, RIFLE criteria positive CAM-ICU.

Re. sample size for secondary endpoints: all these are estimated from binary endpoints, the quantitative sample size calculations contain an additional element of uncertainty (SD). In most cases it will require a much lower sample size to compare quantitatively than binary, if SD is not extreme.

All primary and secondary endpoints will be compared between the two arms, except for time to hypothermia achieved.

3 INVESTIGATIONAL PLAN

3.1 Study Design

Randomized, single-blinded multicenter trial.

3.2 Intervention

560 ICU-patients are included in the study. **All** patients will receive the standardized and recommended diagnostics and treatment used at the specific ICU they are admitted to (*standard of care)*. **Furthermore,** the patients are randomized to:

1. Normothermia (may well have fever) i.e. ***standard-of-care*/control arm.**

or

2. Mild induced hypothermia (target temperature 33 ^{0}C), intended completed within 2 hours after inclusion (maximum core temperature 34 ^{0}C). Hypothermia must be maintained for 24 hours after hypothermia is achieved and subsequently, patients will be heated and kept at normothermia (36 ^{0}C - 38 ^{0}C) for 72 hours from time of enrollment.
   Hypothermia/intervention arm

3.2.1 Information for study participants and guidelines for the investigators. Please see appendix 4 & 5

3.2.2 Recruitment and Randomization

Patients are recruited by the investigator who works at the participating ICU. Advertising will not take place. The randomization is performed by CASS study center and stratified as regards to 1) Study site, 2) age and 3) initial APACHE II score.

Technically, randomization is done by an online computer-based method, which the group also

used for The Procalcitonin And Survival Study (PASS study). All randomization is made in advance and placed in a database hidden behind the online tool. Based on the screening values, the investigator register in the online database, the randomization outcome pulled from the database is used in response to the physician performing the randomization.

The randomization is the result of the online screening conducted. The parameters that are used in the stratification as part of the screening data and must be registered correct for the given result of the randomization is shown (blocking of exclusion criteria and negative responses in the inclusion criteria, respectively). Blocks are used for the randomization (block size are kept blinded so that this information is not compromised).

For patients randomized to hypothermia/intervention arm, hypothermia is aimed to be completed within 2 hours after inclusion. The study coordinator, or his/her substitute (e.g. local principal investigator), must be warned immediately at inclusion and practical conditions are agreed between the study coordinator and the local investigator together with a note on what intervention the investigator and his or hers team are expected to perform, based on the result of the randomization.

3.3 Study population

3.3.1 Inclusion Criteria:

A subject will be eligible for inclusion in this trial only if all of the following criteria apply:

1 Aged ≥ 50 years of age.

2 Severe sepsis[2] /septic shock = →SIRS + suspected infection + hypotension →Mean Arterial Blood Pressure (MAP) <70 mmHg,

3 Admitted to the participating intensive care units (ICU)

4 Indication for intubation

5 Possibility of inclusion within 6 hours after septic shock/severe sepsis is diagnosed in the ICU. Patients admitted with septic shock/severe sepsis should be included within 6 hours after admission. If a patient is not included within this period, that patient cannot be included within the same hospitalization.

6 The patient must have an expected stay in the ICU of more than 24 hours. Anticipated death within 24 hours after admission to the ICU does not exclude participation, however NO decision of narrowing the treatment must have been taken. During this time period,

[2] Since early intervention is presumed to be an essential part of the intervention , it would not be reasonable to to await a possible effect of fluid resuscitation before initiation of the intervention.

probability that the patients is discharged to the master section must not be likely (<10% probability).

3.3.1 Exclusion criteria

A subject will **NOT** be eligible for inclusion in this study if any of the following criteria apply:

1. Pregnant or breast feeding
2. The findings at the initial screening that the patient has a bleeding disorder and/or has an uncontrollable bleeding and/or has had surgery within the last 24 hours
3. Patients who are detained under the Act on the use of coercion in psychiatry

3.3.2 Selection of candidates for participation- discussion of the choice of patients with impaired consciousness

All patients are included acute with subsequent obtaining informed consent from either patient or relatives, as soon as possible. Habile patients will not be included (justified below). Patients with septic shock have a mortality rate about 40%-50% (1). The treatment of these patients in the ICU implies by default that the patient is acutely, upon arrival at the ICU, intubated (connected to a mechanical ventilator) and, in connection therewith, sedated. Therefore, the patient is not able to consider participating in the CASS study.

To make clinical studies, with the goal of improving the treatment of these serious infections, it is necessary to include unconscious patients and patients with blurred consciousness. It is not possible to conduct this study with another (conscious) patient group and get the same result, since infected patients without circulatory failure have a much lower mortality rate and because the pathogenesis of infection without circulatory collapse is very different. Subsequently there will be obtained a written informed consent from the patient himself or, if this is not possible, from close relatives and the patient's family doctor/medical Officer.

The probability of *a priori* to survive with 1) the recommended diagnostic and treatment with the currently available means to identify infections (standard of care) and on the other side by 2) the recommended diagnostic and treatment with the currently available resources to find infections (standard of care) **plus cooling to 33^0 C for 24 hours after the onset of septic shock, must be equal**. This means if during the study evidence arises which can positively determine that one treatment has either a better or worse outcome for patients with septic shock, the study stopped

3.3.3 Reasons for including patients who cannot give informed consent (committee legislation § 13) and for including critically ill, where consent is contained subsequent (committee legislation § 20). Reason C)

"The project can be carried out only by the inclusion of individuals in the particular age group with the disease or condition concerned, and the project is

expected to be of considerable benefit to the group of patients of the same age, with the same disease or condition as the trial subject, and the project entails minimal risk and discomfort for the trial subject"

Adverse effects, potential benefits and drawbacks are discussed in Section 7, *Ethical considerations*. Information on the subject is protected according to Law on processing of personal data and health law. Because septic shock and severe sepsis are stated as acute life-threatening where there is great organ damage for every hour, it is extremely important that a given treatment is initiated rapidly (within minutes to hours) to give the patient the best chance of survival.

Expected improvement of the condition: Bacterial infection is a reversible condition, and if the patient survives because of Mild Induced Hypothermia, the patient's health is expected to improve, usually completely, so that in most cases the patient can resume work, leisure and family life.

Alternative methods of treatment: Currently there are no other treatments before the antibiotics have their full effect on the focus of infection and thereby can reduce bacterial growth plus the ability to form toxins. Simultaneously, hypothermia decreases metabolism, inhibits the initiated "programmed cell death" in a period were the circuit is failing.

Hypothermia a new treatment method that takes its background in the pathophysiological processes occurring during life-threatening infections.

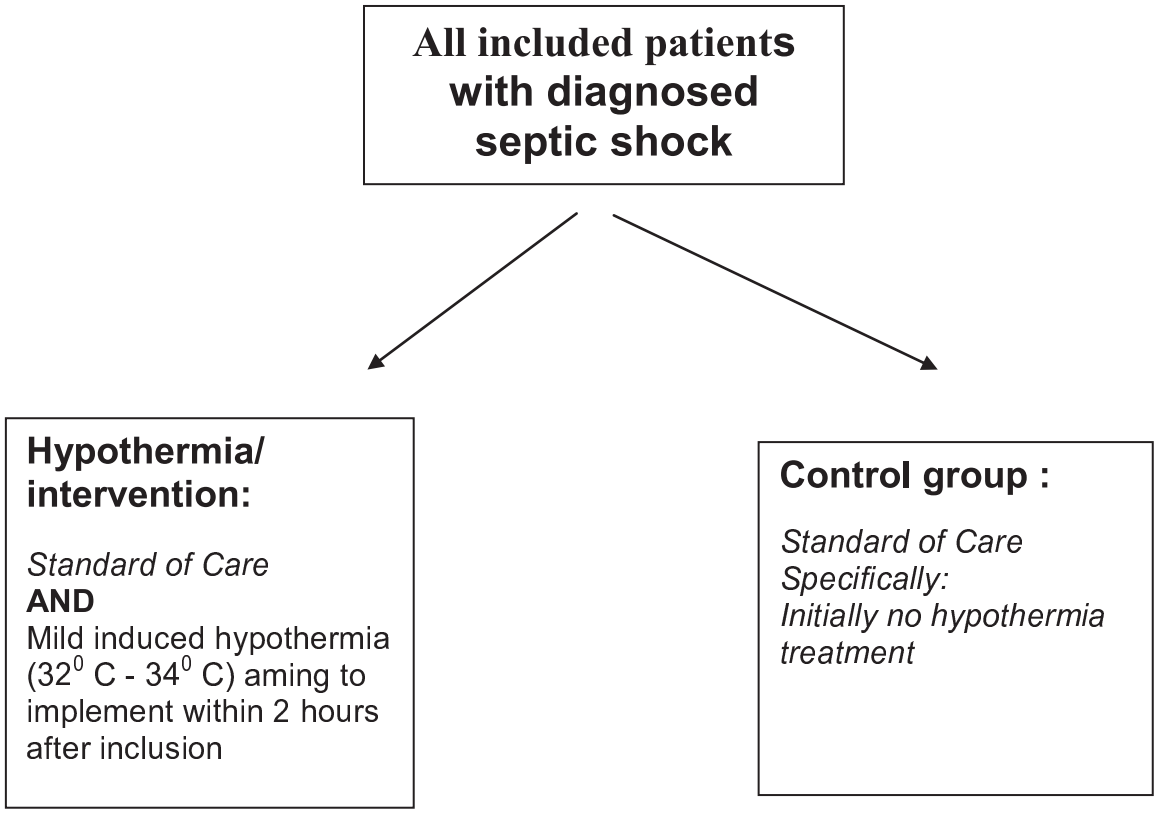
3.4 Treatment during the study

The purpose of Mild Induced Hypothermia is to improve survival by reducing bacterial growth in unsanitized foci (infected areas, where antibiotics cannot penetrate, for example an abscess), dead tissue without vascularisation, when primary antibiotics do not cover the bacterial flora and by reducing inflammatory response by decreasing the enzymatic activity that is heavily involved with the production of cytokines during infection. All patients will receive the usual and approved treatment (standard of care) that is applicable to the specific ITA.

It is obligate that the participants in both the control and intervention group have received the first dose of antibiotiotics maximum 1 hour after septic shock was diagnosed. Empiric treatment (unknown microorganism) in both groups must meet the minimum of piperacillin / tazobactam + ciprofloxacin or alternatively meropenem monotherapy. In case an abdominal focus and/or aspiration cannot be excluded, metronidazole is added. Of the acute drugs, the most broad-spectrum is administrated first (e.g. in case of administration of; Meropenem + ciprofloxacin + metronidazole, starting with Meropenem, then ciprofloxacin and lastly metronidazole).

The CASS study group will in consistency with the protocol create guidelines for all interventions that must be conducted by randomization to the intervention group. Some variations between the

various participating ICUs are acceptable, whereas the diagnosis and treatment of each ICU should follow the guidelines for intervention used by the particular department. In the local guidelines for intervention, may well exist several alternatives indicated for a given situation. "Investigator" is not obliged to follow these guidelines, if he or she otherwise is complying with the protocol.



- **Severe sepsis/septic shock defined as (all 3 criteria must be present)**
  - **Low blood pressure → Mean Arterial Blood Pressure (MAP) <70 mmHg.**
  - **Systemic Inflammatory Response Syndrome (SIRS)**

    → 2 of the following 4 clinical parameters:

    A) Temperature ≥38,0⁰ C or ≤ 36,0⁰ C

    B) Heart rate ≥ 90 beats per min.

    C) Respiratory rate ≥ 20 per min. or $PaCO_2$ < 32 mmHg

    D) WBC ≥ 12 mia/ L or ≤ 4 mia / L or bandemia ≥ 10%
  - **Infection**

3.5 Changing the strategy during the study

For patients randomized to the intervention group, the intervention can only be stopped if the ratio between benefits and risks of the intervention are not acceptable for the treating physician. The specific concern of the treating physician must be reported to the CASS coordination center, which in some cases after consulting the CASS steering committee, is responsible for interpretation and possible action if necessary.

The intervention can also be stopped if the patient /relatives decide that the participant should be removed from the study.

3.6 Modification of treatment in the control arm (*standard of care*)

If patients in the control arm, should be in the need for treatment with hypothermia (according to *standard of care guidelines*) while they are participating in the CASS study, e.g. heart failure at some time during their hospitalization, hypothermia should be implemented if it is otherwise indicated and not contraindicated. This is defined as *standard of care.*

This does not mean that the patient is excluded from the CASS study c.f. *intention to treat analysis* will be conducted.

3.7 Vassorpressor/inotropic and dosages

All medicaments affecting circulation to increase blood pressure, "vasopressor" substances such as Dopamine, Dobutamine, Noradrenaline and Adrenaline, which is prescribed during hospitalization must be registered in the medication schedule regarding dose, date and time of prescription and discontinuation. Handling of toxicity is done by guidelines as mentioned below and applies to all components of the study.

3.8 Use of antipyretic

Patients in the control arm may not receive antipyretic drugs at any time during the study.

3.9 Toxicity

In case of adverse effects, therapy with vasopressor agents can be interrupted by involving the "investigator" and taking into account the severity of the adverse reaction. The dose can be reduced, suspended or re-introduced depending on the current knowledge about the adverse effects and depending on the severity of the adverse reaction. Patients who require a dose reduction should be re-evaluated daily.

"Investigator" is responsible for taking the right precautions to ensure that the risk of developing toxicity is minimized, the patient is monitored for the development of toxicity, its toxicity and if they occur, to take the right steps to minimize their impact.
A biobank will be created during the CASS study. The study group already has a biobank consisting of 1000 patients from the PASS study.

3.10 Biobank

A research biobank is established in the form of whole blood. The biobank is established to promote research on treatment of severely infected patients with circulatory failure (septic shock). Overall, there will be conducted hypothesis-generating analysis, and testing of these with the development of diagnostic models based on genetic variations (gene markers).

Methods: In connection with daily blood sampling, 15 ml of whole blood is taken for use in rutine diagnosing and treatment of the patient. The amount of blood that are not used immediately (about 7 ml), is stored in a research biobank for later analysis. Personal information is protected under the Privacy Act. Participants are assigned a participant number and hereby become anonymous, whereby the stored blood plasma and DNA is stored in anonymous form. Material will not be passed on to others or taken out of the country and, will be destroyed when the research project is complete. Creation of research biobank is reported to the Data protection Act (DPA) (journal number 2011-41-6470).
Plasma Proteins: There will be special emphasis on proteins involved in coagulation, fibrinolysis and epithelial activation because the hypercoagulative state with blood clot formation and circulatory failure plays a crucial role in the development of septic shock (4). Core proteins that are being investigated are Plasminogen Activator Inhibior-1 (PAI-1), Tissue Factor, Tissue Factor Pathway Inbihitor, Syndecan-1 and thrombomodulin.
Analysis of the above proteins and their correlation with the severity of organ and circulatory failure in the patient group are made. Subsequently, these analysis are supplemented by studies of genetic variations (in the form of single nucleotide polymorphisms, SNPs) and their potential for predicting the prognosis of septic shock. Focus will be on SNPs with a frequency of at least 10%, and has a described relationship with activity or concentration in plasma of the protein, such as 4G/5G variation in SERPINE1 encoding for PAI-1(48).
Laboratory tests: ELISA, TaqMan ® RT-PCR and Next Generation Sequencing.
Power Calculation: With 560 patients and a mortality of 56% (own data), a 80% power is achieved to detect a hazard ratio of 2.0 for death of alleles (determined DNA sequence) which is found in more than 10% of population.
Ethical considerations: Because the research biobank is using excess material from routine blood tests the patient will not be exposed to additional stress or risk associated with sampling.

The collected material will not affect participation in the main project or have consequences of treatment in connection with this or future admissions for the patient themselves or the patient's relatives.

Perspective: On the basis of the research biobank treatments causing serious side effects such as organ failure (specific broad-spectrum antibiotic) can possibly be avoided by tailored management of the patient with background in the genes.

Information relating to the study participant regarding research biobank and consent to donate biological material: The participants are getting specific information regarding the collection of material for a research biobank, where the above-mentioned conditions are clear. It is emphasized that participation in providing biological material does *not* affect participation in the main project (hypothermia treatment) and the donor can decide if he/she is interested in getting information about results. The donor must sign a separate consent form.

3.11 Investigator Manual

Please go to appendix 5

3 ECONOMIC RELATIONSHIPS

Patients will not receive any economical compensation for participation.

1. Initiative: A) Physicians at the participating Intensive Care Units, B) Scientists, including doctors at the Coordination Centre, Copenhagen HIV Programme (CHIP) which is an organization under the University of Copenhagen, Faculty of Health Sciences and Clinical Microbiology Department, Rigshospitalet, C) Doctors at the Clinical Microbiology Department. at Hvidovre University Hospital
2. Applications for funding are ongoing and currently The CASS Project Group have received 1 million Dkr. from the Lundbeck Foundation, and 2 million Dkr. from Trygfonden. Furthermore an application to Forskningsråd for Sundhed og Sygdom (FSS) is planned and in addition to, Fonden til Lægevidenskabens Fremme (A.P. Møller), Harboefonden, and several other foundations. The research group has itself sought and obtained donations equivalent to 5.8 million Dkr for *The Procalcitonin And Survival Study*, which were finished via 8 intensive care units in Denmark. The main costs will derive from scientific management (project coordinator),project nurse system, and monitoring and data handling
3. Investigators are not linked to private companies. All project staff are employed in public hospitals or at the Health Sciences, University of Copenhagen

4 MESUREMENTS AND EVALUATION

5.1 Time and evaluations schedule

A flow chart showing the timing of study procedures (Clinical and Laboratory) is shown in table 4.

An initial pre-entry (screening) assessment for eligibility will be performed as soon as possible after the patient is admitted to the ICU. The patient should be randomized no later than 6 hours after the time of admission. Evaluations will then be carried out at entry (Day 1), and thereafter daily as long as the patients remains in the ICU (or deleted from the study of another cause). After discharge, the course of disease is collected in less detail and the survival status determined day 30, 60, 90, 120 and 180 after enrolment in the trial.

5.1.1 Pre-entry evaluation

The pre-entry evaluation will be conducted the first day of the study (day 1) by an investigator at the ICU and will include an evaluation of whether the patient fulfils the requirements for enrolment in this trial. Subjects who fail to meet the entry criteria may not be re-screened for this protocol until 30 days after the failed pre-entry evaluation. Hence, enrolment of such patients will require that the patient is re-admitted to the ICU after at least 7 days outside of the ICU after the time of the first screening.

5.1.2 Baseline evaluations (Day 1)

The following evaluations should be performed at baseline (Day 1):
Note: For this trial, Baseline (Day 1) is defined as the day on which the patient is included in the CASS study via online randomization. The following data are to be collected on day 1:

- Demography including date of birth, weight, height, and indication for admittance to the ICU
- Infections found in the subject in this hospital admission prior to admittance to the ICU.
- Present infection focus/ etiologic microorganism
- APACHE II score (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale)
- Dosage of inotropic agents, both maximum in recent days and six o´clock-value.
- Current medical conditioning (including if the patients is receiving dialysis)
- Pre-admittance daily function and health state:

Professional career:	1) Student, 2) Part time work, 3) Full time work, 4) Early retirement, 5) Retired
Health:	1) Congenital handicapped, 2) Acquired handicap, 3) Chronic disabling disease, 4) Chronic non-disabling disease, 5) Healthy

Self-supportance:	1) Lives in nursing home, 2) Lives in a flat connected to a nursing home, 3) Own home with external help ≥ once / day, 4) Own home with external help < once daily, 5) Own home, no help required
Hospital need:	1) ≥ 3 months admitted to a hospital/ last year, 2) 1-3 months admitted to a hospital/ last year 3) 1-30 days admitted/ last year, 4) No admissions, ambulatory visits ≥ 6/ last year, 5) No admissions, ambulatory visits 1-5/ last year, 6) No admissions, No ambulatory visits/ last year

- Adverse events/ other complications to treatment given in this hospital admission (ongoing clinical conditions at Day 1 must be recorded in the "Adverse Event and Medical Condition Form" of the CRF at this time, regardless of the fact that such conditions may not subsequently be found to fulfill the definitions for an adverse event

- Hematology: *hemoglobin, platelet count (WBC count mentioned as part of APACHE II)*

- Clinical chemistry: Albumin, Bilirubin, Factor 2-7-9, Alanin Aminotransferase (ALAT)/ Aspartate Aminotransferase (ASAT), Alcaline Phosphatase, Creatinine, Carbamide, Na^+, K^+, Phosphate, Ca^{2+}, C-reactive protein (some are also mentioned as part of APACHE II).

5.2 On trial evaluations

On trial assessments will be completed at the following time-points unless otherwise specified:
While admitted to the ICU, the following information will be registered unless specified otherwise:

Daily while patient is admitted to the ICU:

- Mean Arterial Pressure (systolic and diastolic blood pressure)
- Inotropic agents, both maximum infusion rate and a.m. 6.00 value.
- Clinical signs of a new (nosocomiel) infection
- Microbiological or radiological evidence of a new (nosocomiel) infection
- Defined daily doses of antibiotics
- Sequential Organ Failure Assessment (SOFA) score (PaO_2, FiO_2, patelets count, Systolic and diastolic BP, amount of vasopressor agents (adrenaline, noradrenaline, dopamine, dobutamine), Glasgow Coma Scale, Bilirubin, creatinine)
- Presence of sepsis, severe sepsis, septic shock, Disseminated Intravascular coagulation (DIC). Assessment of cerebral status by RASS and CAM-ICU, Blood pressure (systolic blood

pressure < 90 mmHg), days of mechanical ventilation, Factor 2-7-9 < 0.7, creatinine (increased by a factor of 3 from starting point), Multi Organ Dysfunction Syndrome (MODS).

- Adverse effects/other complications to the treatment given at the ICU (clinical conditions / illnesses on day 1 is registered in "Side effects and disease section" in the CRF (Case Report Form = Individual registration sheet for each patient participating in the study) at this time, although such conditions sometimes prove not to meet the criteria of an adverse event (see side effects section for definitions).
- Hematology: Hemoglobin, Platelet count and WBC
- Biochemistry: Albumin, Bilirubin, Factor 2-7-9, Alanine Aminotransferase (ALT)/aspartate aminotransferase (AST), alkaline phosphatase, Creatinine, Carbamide, Na^+, K, Phosphate, Ca^{2+}, C-reactive protein (CRP)
- Diagnostic imaging procedures performed
- Microbiological sample taken
- Surgical procedures performed (please see section 6.3.1)
- Diagnostic imaging findings that have been performed
- Microbiological tests performed
- Surgical procedures performed
- Changes in antibiotic treatment
- Consumption of SAG-M in the recent 24 hour
- Time of beginning and type of Dialysis (noted in the CFR)
- Glascow Coma Scale (GCS) day 1

At the day of discharge from ICU, the later pre-defined dates or the day of death:

- Information regarding death, time and course
- Discharge and post discharge daily function and health to day 30, 60, 90, 120, 180.

5.3 Study drugs

Drugs with a background in the study will not be prescribed, but patients will receive relevant medicine, as required according to the *standard of care* to treat their diseases in the best way. This will occur in both the control group and the intervention group and will exclusively be controlled by the attending physician's prescriptions and NOT of interventions in the CASS study.

5.3.1 Information regarding dosage

The following information on dosage of all prescribed inotropic agents and antimicrobial drugs during the study must be registered in the "Medicine" section of the CRF.

- Date of prescription
- New dose after change, along with reason for change
- Date for discontinuation for each drug

- Reason for discontinuation
- Date of re-ordination

6 DATA ANALYSIS METHOD,SAFETY,STUDY MONITORING AND INTERRIM ANALYSIS

6.1 ”Sample Size” determination

The trial will randomize (1:1) 560 patients into two arms:

1: Control arm

2: Hypothermia/intervention arm

The sample size is estimated from following assumptions:

1. A presumed presence of the primary endpoint of 56% in the control group (estimated from data from patients admitted to the participating departments with diagnoses septic shock in year 2008-9.).
2. A detection limit of relative risk for the primary endpoint of 0.79 (21% relative reduction in mortality).
3. Strength to avoid type II error (power) of 80% (1-beta).
4. Limit for Type I error of 5% (Should alternative hypothesis be confirmed, there will be <5% chance that this is a random finding with the observed distribution).

The sample size is also sufficient to examine the hypothesis if the control group mortality is 40% (instead of 56%). In this case with detection limit of relative risk of 0.72.

6.2 General Considerations

Definition of time perspective

Day 1. = Baseline. Registrations are made as stated before induction of hypothermia or if not specified in the period 0-6 hours.
Day 2.= 24 hours, if not otherwise specified
Day 3.= 48 hours, if not otherwise specified
Day 4.= 72 hours, if not otherwise specified

6.2.1 Population for analysis

The primary population for analyses of the efficacy and safety data will be the “intention to treat” population, including all randomized patients.

6.2.2 Primary Endpoint

The primary efficacy analysis will be the comparison of the two treatment groups with respect to the incidence of mortality within 30 days after enrollment in the trial. Mortality is defined as all-

cause mortality.

6.2.3 Secondary Endpoint

1. Duration of cardiac/septic shock:	A) Delta Mean Arterial Pressure (MAP). Analysis: MAP (day 4, a.m. 4:00 to 9:00) – MAP (day 1). B1) Inotropic score day 1, day 2, day 3, day 4 (time 0, 24, 48 and 72 hours); = (dopamine doses x 1) + (dobutamine doses x 1) + (adrenaline doses x 100) + (noradrenaline doses x 100) + (phenylephrine doses x 100), doses given in µg/kg/min. B2) Inotropic score day 1+day 2 + day 3 + day 4 (time 0, 24, 48 and 72 hours) (accumulated need for inotropic) C) Achieved discontinuation of vasopressor/inotropic on day 2, day 3, day 4 (a.m. 4:00 to 9:00). SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 50%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,25 (requires 480 patients)
2. Respiratory failure:	A) Delta PaO2/FiO2 ratio. Analysis: PaO2/FiO2 ratio (day 4, a.m. 4:00 to 9:00) - PaO2/FiO2 ratio (day 1) B) Achieved discontinuation of mechanical ventilation on day 4 (a.m. 4:00 to 9:00). SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 40%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,29 (requires 560 patients)
3. Renal failure:	A) Acute renal failure cf. the RIFLE criteria. Analysis: 1) Increase in serum-Creatinine at x 1.5 at any time from day 1 = time of randomization before induction

	of hypothermia and day 4 (a.m. 4:00 to 9:00) OR decline in eGFR of> 25% (both providing endpoint). Analysis 2) Increase in serum-Creatinine at x 2.0 at any time from day 1 = time of randomization before induction of hypothermia and day 4 (a.m. 4:00 to 9:00) OR decline in eGFR of> 50% (both providing endpoint). Analysis 3) Increase in serum-Creatinine at x 3.0 at any time from day 1 = time of randomization before induction of hypothermia and day 4 (a.m. 4:00 to 9:00) OR decline in eGFR of> 75% OR serum-Creatinine ≥300 μmol/L (all three providing endpoints). SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 30%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,38 (requires 560 patients)
Cerebral:	A) Delta Richmond Agitation Sedation Scale (RASS) Analysis: RASS (day 4, (a.m. 4:00 to 9:00) – RASS (day 1) B) NEGATIV Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) 24, 48 and 72 hours after Awakening. SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 60%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,19 (requires 560 patients)
5.Hepatic:	A) Delta Bilirubin. Analysis: Bilirubin (day 4, (a.m. 4:00 to 9:00) - Bilirubin (day 1) B) Achieved Bilirubin> = 21 mM at 72 hours (Day 4) SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint:

	Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 20%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,5 (requires 560 patients)
6.Coagulation:	A1) Delta platelets. Analysis: platelets (day 4, a.m. 4:00 to 9:00) – platelets (day 1) A2) Delta platelets/binary: Achieved decrease in platelets >25% day 1 → day 4. B) Delta INR. Analysis: INR (day 4, a.m. 4:00 to 9:00) – INR (day 1) C) Delta Factor II, VII, IX (”PP”). ”PP” (day 4, a.m. 4:00 to 9:00) – ”PP” (day 1). D) Delta APTT. APTT (day 4, a.m. 4:00 to 9:00) – APTT (day 1) E) Total consumption of SAG-M the first 10 days F) Occurrence of severe bleeding (1) CT confirmed ICH, 2) fresh upper or lower gastrointestinal bleeding 3) other bleeding with need for surgery). G) Trombelastrography (TEG) or tromboelastrometry (ROTEM) is executed in the departments who have the opportunity. (Time R/CT (s)) Angel, MCF/MA. Day-to-day variability (unilateral variation analysis: ANOVA) Lysis30 min (lysis 30 min after maximum amplitude) SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 35%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,33 (requires 560 patients)
7.Infection:	A) Quantitatively: Delta C-reactive protein (CRP). Analysis: CRP (day 4, a.m. 4:00 to 9:00) – CRP (dag 1) B) Binary: Achieved decrease in CRP >30% from day 1 →

	day 4. SAMPLE SIZE: Corresponding sample size/power for the assessment of this endpoint: Type I limit 0.05, type II limit 0.2 (power 0,8) Based on endpoint C at 72 hours (day 4). Paragraphs: endpoint was achieved in the control group at 35%. Limit for reliable detection of relative difference (both ways) to the occurrence of endpoint in the hypothermia group: 1,23 (requires 560 patients)
8. Days without organ failure up to day 30	Organ failure is defined by: the need for mechanical ventilation, need for inotropic, RIFLE criteria positive CAM-ICU.

Re. sample size for secondary endpoints: all these are estimated from binary endpoints, the quantitative sample size calculations contain an additional element of uncertainty (SD). In most cases it will require a much lower sample size to compare quantitatively than binary, if SD is not extreme.

6.2.4 Other factors

All patients will, when possible, participate in the study and will be followed until 180 days after inclusion.

6.3 Effect

6.3.1 Descriptive analysis of the hypothermia group and the control group

The hypothermia and control group are designed to show diverse physiological profiles incl. varying oxygen demands and a varying incidence of organ-related complications of septic shock. If these differences do not occur either in the physiological profiles or in the organ-related complication rate, it would be extremely unlikely to find differences regarding the primary endpoint. As a result, the ongoing monitoring of the study beyond safety (described below) will focus on these differences, besides the primary endpoint in itself.
A detailed description of the ongoing monitoring parameters found in Section 6.4 Safety. Changes ("delta" from the baseline to day 4) in the core parameters concerning organ functions incl. MAP and FiO2 will be analyzed continuously (details in section 6.4 Safety). Median time to discharge from ICU and from hospital will be analyzed.

Besides the above mentioned primary and secondary endpoints the study will analyze:

1. Time to broad spectrum antibiotic (minimum cf. Meropenem or

Piperacillin/Tazobactam+Ciprofloxacin).

2. Time to extubation
3. Sequential Organ Failure Assessment (SOFA) score (PaO_2, FiO_2, patelets count, Systolic and diastolic BP, amount of vasopressor agents (adrenaline, noradrenaline, dopamine, dobutamine), Glasgow Coma Scale, Bilirubin, creatinine)
4. Occurrence of secondary infections verified within 10 days after recruitment. (Secondary infection is defined as: clinical signs of infection after the patient is assessed clinically uninfected at least 2 consecutive days. The infection must be verified microbiologically or radiological.)
5. Number of surgical procedures after recruitment.
   Surgical procedures are defined as invasive procedure which is not:
   - Various entries (arterial cannula, CVC, venflon, epidural catheter)
   - Nephrostomia
   - Dialysis
   - Suprapubic catheter
   - All types of drains
   - Invasive ventilation incl. tracheotomy

For the hypothermia group, the following is also analyzed:

6. Time to hypothermia, meaning time to Tp < 34 °C.
7. Procalcitonin

Endpoints that are normally distributed data series will be evaluated statistically by using t-test and when the endpoints are not normally distributed, as Mann-Whitney. Categorical parameters are estimated using Chi Square for equal proportions.
It is expected based on hypothesis, background and design that one or more organ-related complications will have a lower incidence in the group receiving hypothermia treatment (measured through the secondary endpoints described above).

<u>Furthermore the groups receiving treatment is compared in subgroup analysis, defined as groups of patients:</u>

A) APACHE II score ≥ 25 vs. <25
B) Age≥65 year vs. < 65 year.
C) Renal Failure at baseline (eGFR <60ml/min/1,73 m2 vs. ≥ 60 ml ml/min/1,73 m2)
D) Surgical vs. Medical patient (defined as the ward the patient is moved from to the ICU)
E) The ICU the patient is hospitalized at
F) Calendar period for randomization (1. half vs. 2. half)
G) Gender

6.3.2 Analysis of Primary and Secondary endpoints

The main analysis of the primary endpoint will be an “intention-to-treat” analysis by comparing the two treatment groups with regard to mortality within 30 days after inclusion.
Mortality is defined as death from all causes. Patients who cannot be followed for 180 days (as specified in the protocol), *lost to follow-up,* will be counted as survivors. It is expected that only very few patients cannot be followed in the indicated period, because of the Civil Persons Registration (CPR), in which all deaths of Danish citizens and persons with permanent address in Denmark are registered. Only patients who permanently relocate their address to another country within 30 days after ITA hospitalization can be *lost to follow-up* with respect to the primary endpoint. The primary endpoint will be analyzed both as a categorical parameter (alive on day 30 after inclusion) and as a quantitative parameter (the day of death, "time to event") using "Cox proportional hazard” regression model and graphically as Kaplan-Meier cumulative "event" curves to summarize results concerning the primary endpoint.
Stratification in the subgroup analyzes will be performed in the same groups, as mentioned above in the descriptive analysis.
The secondary endpoints are chosen based on key criteria that they must 1) be able to describe the patient's progress and a potential effect of hypothermia treatment based on background and hypothesis, 2) possibly be a "precursor" or a "warning" on the primary endpoint.
Details of the selected secondary endpoints are described in Chapter 2 of "Purpose and Outcome."
The analyzed levels of significance are corrected in light of the repeated interims analysis according to the O'Brien-Fleming and Pocock models. The significance limits on the different interim analysis shown below in *section 6.3.4*. regarding *DSMB and interim analysis*.
If patients are withdrawn from the study prematurely censoring from the stop-time will occur (relatives to unconscious patients or patients who wish not to participate).

6.3.3 Analysis of coagulation status during extraordinary interim analysis

All participants in both the hypothermia group and the control group will have coagulation parameters measured on a daily basis according to *Section 5.2*. Furthermore, the first 50 patients (minimum 25 patients per group) will get extensive coagulation status measured consisting of different blood samples plus undergo coagulation analysis on whole blood by trombelastography (TEG) or troboelastrometry (ROTEM) during the first 3 days.
TEG / ROTEM Analyses are conducted on whole blood at the patient's *actual body temperature* i.e. the blood is analyzed at the same body temperature as the patients temperature at the time, and at 37 °C.
Blood samples are drawn; day 1 (before hypothermia treatment initiation), day 2 and day 3 (immediately after rewarming to body temperature above 36.5 °C). Coagulation tests (INR,

Platelet and APTT) will be measured day 7 and day 10 Furthermore, the consumption of SAG-M within the first 10 days is recorded.
Analysis of coagulation are collected and submitted to the coordination center. These data will be used for the planned pause and interim analysis following respectively after inclusion of 10 and 50 enrolled patients (see below).

6.3.4 Data and Safety Monitoring Board (DSMB), Interim analysis and other study monitoring

Besides this section, please refer to section 6.2.2, 6.4 and section 7

Planned analysis of safety and efficacy data will be assessed when 100, 250 and 400 patients have completed the study (until hospital discharge or death). These assessments will be made by an independent Data and Safety Monitoring Board (DSMB). A specific date will be chosen for each of these analysis based on current enrollment rates, and all data on primary and secondary endpoints and any adverse event data before that date will be used.
In addition, the Data and Safety Monitoring Board has the possibility to require an extraordinary interim analysis at any time. In section 6.4,Safety, is described in details what data, in particular can form the background for such an extraordinary interim analysis.
Significance testing will be subject to assessments, among others, based on O'Brien-Fleming and Pocock plots, to test differences in treatment outcomes in interim analysis.

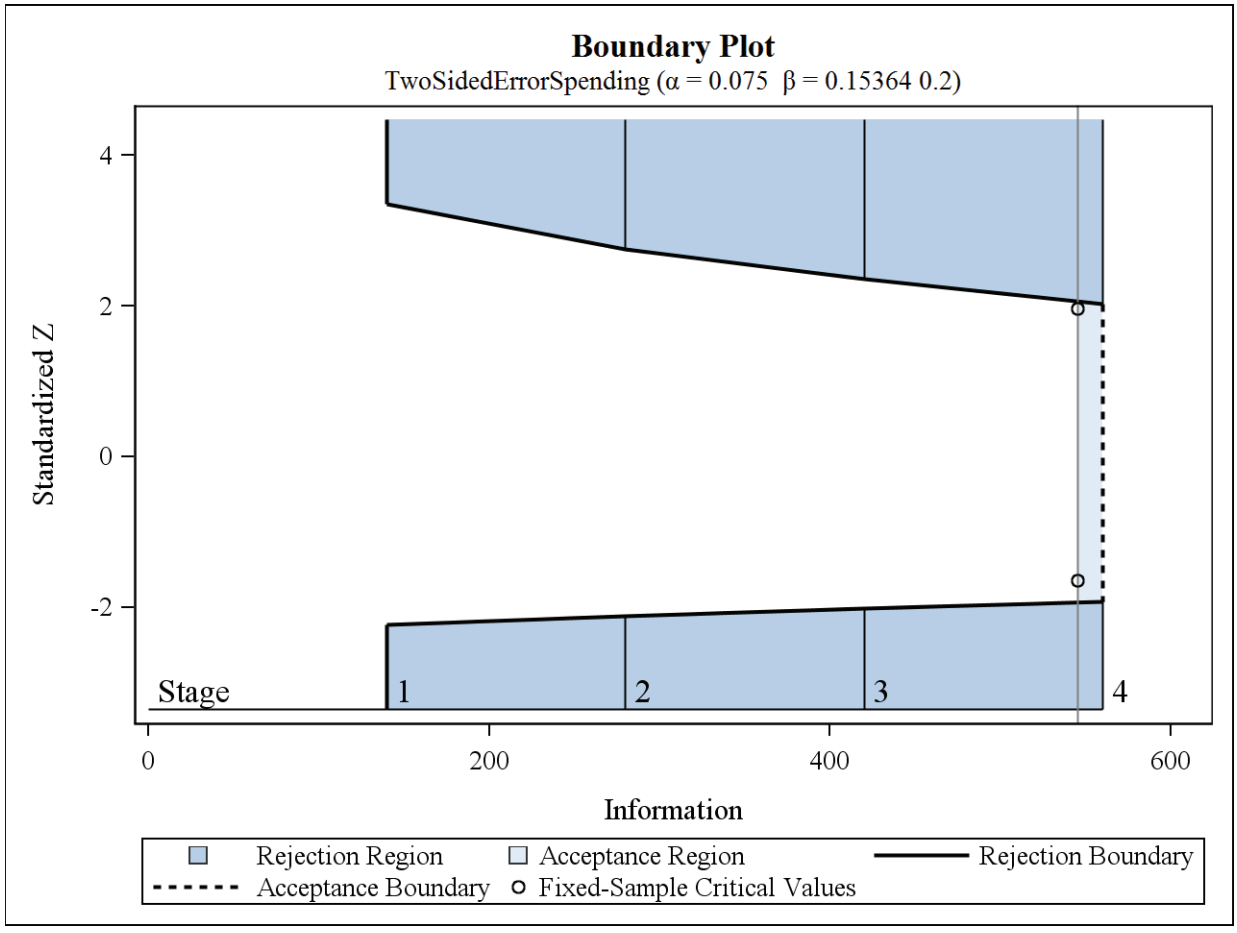
6.3.4.1 Rules for stopping " The Cooling And Surviving Septic shock" study

Boundary values have been derived using the Lan and DeMets approach using an error spending function to specify the errors at each stage for each boundary. We designed a 3-stage two-sided equally spaced asymmetric group sequential design for normally distributed statistics. It is asymmetric because it has an O'Brien-Fleming upper boundary and a Pocock lower boundary; the latter approach is known to be less conservative and should insure earlier stopping if mortality is higher in the intervention arm compared to standard of care. The O'Brien-Fleming boundary was approximated using a power family error spending function with parameter rho=3, and the Pocock boundary with parameter rho=1 [Jennison and Turnbull].
In this study, we plan to recruit a total number of 560 patients; planned interim analysis will be made at inclusion of 140, 280, and 420 patients. The final main analysis, with the total data, will be conducted when all 560 patients are included; at this analysis, the overall limit of significance at 0.05 be applied. Therefore, under the assumption that the planned number interim analysis is 3 and type I error alpha = 0.05 the following table is obtained:

Interim analysis	Number of patients recruited	Normal deviate Z-value (upper boundary)	Normal deviate Z-value (lower boundary)
1	140	3.359	-2.241
2	280	2.760	-2.125
3	420	2.359	-2.019

Table 3: limits of significant at interim analysis cf. the O´Brien-Fleming and Pocock models

Interpretation: If the z-value for mortality analysis is larger than the upper boundary value at the specified interim analysis (i.e. the test is in the upper rejection region) the trial may be prematurely stopped because the intervention is superior to standard of care. In contrast, if the z-value for mortality analysis is smaller than the lower boundary value (test is in the lower rejection region) the trial may be prematurely stopped because the intervention is inferior to standard of care. The values in Table 3 are shown in the plot below (darker shaded areas indicate rejection regions).



A premature study closure will not be based on a statistical background alone, but on the recommendations of the DSMB. However, it will be of paramount importance for the DSMB, if the mortality data cross these limits, especially if the lower limit. Regarding other "acute" or non-acute collected data for the interim analysis, the DSMB will evaluate these as a whole and always facing

mortality data and these in relations with the above principles, models and limits in Table 3.

Besides the planned interim analysis it will be possible for the DSMB to require extraordinary interim analysis´ *at any time*.

The primary purpose of this study surveillance is to monitor and 1), provide patient safety, but also 2) "futility" (the study's ability to clarify the purpose), 3) effect, 4) the interventions completion and 5) the inclusion rate will be monitored by interim analysis. In addition, the DSMB monitoring study scientific quality overall (data collection, quality of data, the export of study-related procedures, any methods of blinding and deviations, etc.).

Re.: 1) Safety: Please see Sections 6.4 and 7.0

2) “Futility”. Please see Section 6.3.1 and 6.3.2 for the parameters that will form the basis for "Futility" analysis. The basic idea is that the patient parameters, as it is documented have an impact on the later survival and that the hypothermia procedure is expected to have a positive physiological effect against (primarily organ function and infection status), especially in terms of cerebral function, cardiovascular function, respiratory function and renal function will be assessed on dynamic (i.e. changes in the functional level over time during the ICU hospitalization.)

3) Effect: Effect analyses are described in 6.3.1 and 6.3.2.

4) Interventions (Adherence): To monitor the quality of an intervention study it is crucial to assess whether the intervention is carried out correctly as described in the protocol in all patients in the intervention group. This will be done with a primary analysis, which reports the proportion of patients who have achieved a core temperature of 32 ^{o}C<Tp<34 ^{o}C within 2 hours and have maintained this temperature for min. 24 hours.

5) Inclusion rate: At least 560 patients at 8-12 intensive care units in Denmark must be included. Therefore, 45-70 patients per. department must be included. The planned participating ICUs, which are liable to be the largest in the country, are the same as that where participating in our previous intervention study. Each of these departments admits annually 50-80 patients with septic shock. By inclusion of all these departments the inclusion period will therefore be 6-12 months. Based on our experience around 50% of the suitable candidates are included, and inclusion period is therefore expected to be around 12-24 months, or correspondingly shorter, if so decided by the steering committee that more departments are to be invited (Science Ethics Committee requested in this case authorizing the enlargement of the area). This corresponds to inclusion of an average of approx. 20-25 patients monthly the entire study period. Our experience from our past study is that inclusion speed will double in the second half of the study period. It

is therefore expected that after the first 12 months 190 participants are included. If the actual figure is well below this minimum, it should lead to a consideration of whether the study can increase enrollment rates significantly and, if not deemed possible, the study must close for this reason.

The DSMB may at any time ask for there to be made other analysis either as single parameters or composite parameters if deemed necessary in order to monitor the study.

Members:

Before enrollment of the first participant in the CASS study, the DSMB is established . The DSMB will be in line with previous studies from the group and will consist of 3-5 persons, which have the following qualifications: a) high scientific integrity and production, b) extensive experience with randomized controlled trials and safety considerations for these, c) The CASS study group must have great confidence that these persons will safeguard the interests of the patients and the study in the most fair way.

Additionally, a least 2 members must have extensive knowledge of intensive care patients and bacterial infections.

CASS Advisory Group will suggest that the following candidates included:

1. Henry Masur MD, Chief, Critical Care Medicine Department, Clinical Center, DHHS Bethesda, Maryland, National Institutes of Health, + Editor at *Critical Care Medicine (Society of Critical Care Medicine)* USA
2. Christian Torp-Pedersen, MD, DMSc, professor of Cardiology, Gentofte Hospital (Copenhagen University Hospital)
3. Andrew Copas, PhD, senior lecturer in Medical Statistics, UCL Research Department of Infection and Population Health, Royal Free Campus, London
4. Court Pedersen, MD, DMSc, professor of infectious diseases, Department of Infectious Diseases. Odense University hospital.

Plan:

Scheduled interim analysis at enrollment of 100, 250 and 400 patients. In addition, the DSMB can at any time (given the conditions mentioned in section 6.4 or other conditions which DSMB finds appropriate) asses any data in the database and also require that additional data is collected, if necessary in order to investigate if patient safety is dealt with in the best way.

Monitoring and Quality assurance of data

In accordance with applicable Good Clinical Practice (GCP), the monitors periodically contact the department where the study takes place, including an appearance on the ward. The extent, form

and frequency of attendance on the ward will be determined based on enrollment rates, quality of data and documents that the department comes up with, the consistency of follow-up of patients under this protocol. During these contacts (telephone / writing / reporting to) the monitor will:

• examine and assess the progress of the study
• quantify and evaluate the collected study data
• perform the "Source Document Verification"
• identify any problems and seek their solution

This will be done to verify that:

• data are authentic, accurate and complete
• patient safety and rights are protected
• the study conducted in accordance with the currently approved protocol (and any amendments approved by the projects coordinator), GCP and all other applicable rules

"Investigator" agrees to allow the monitor to access all relevant documents and to spend the requisite of his/her time as well as his /her staff time for the monitor to carry out controls and discuss the relevant findings during inspections.

Besides contact during the duration of the study, the monitor also contacts the department prior to the beginning of the study to discuss the protocol and data collection procedures with the department staff. On completion of the monitor will carry out all activities as mentioned in Section 7.5, Study and study department closures (Trial and Site Closure).

6.4 Safety

6.4.1 Acute Safety Monitoring

In order to monitor patient safety, patient for patient, there will in addition to the normal data-collection be collected data on death, organ involvement and blood coagulation from a separate registration and reporting plan. Mortality Reports must be submitted to the coordination center within *72 hours* after death. The investigator may contact the coordination center if he/she suspects that the intervention is harmful.

<u>Reporting of any deaths occurring during intensive hospital:</u>

Any death in intensive care hospitalization in both groups will be followed up by a report on a separate recording sheet, where the investigator in collaboration with the treatment team is asked to assess the cause of the current death. There will be 1) a part with check boxes and 2) a free text-part.

In the “check box-part” the causes of death will be divided into 1) Key reasons, 2) Reasons which may be involved 3) Reasons which are possible but unlikely to be involved.

1) The data which will be included in the “check box part” will be categorized in the above 3 categories and will be: A) refractory septic shock, B) cardiac dysfunction, C), renal dysfunction, D)

Respiratory dysfunction, E) cerebral dysfunction, F) hepatic dysfunction G), coagulopathies incl. bleeding, H) immunological dysfunction, I) chronic diseases incl. innate, J) other reason (briefly described and elaborated in free text part)
2) Besides the "check box part" where causes of death are categorized as above, the investigator will be asked to describe in his/her own words, which particularly conditions prevailed for the reported death.
The actual death is recorded in the database within the first weekday after the report is received.

Reporting of deaths suspected directly due to the intervention
If the investigator estimates that a death is directly caused by the intervention, this suspicion should be telephoned immediately (same day) to the project coordinator. In consultation with the steering committee it is decided whether the study should be paused. A pause will lead to a call for the DSBM and the enrollment is only resumed if the DSMB (and steering committee) considers this to be safe.

Reporting of deaths occurring after discharge from the ITA, but within 30 days of enrollment
Once monthly follow up on all patients included in the CASS study in the past month. All deaths in these patients are registered in the database (the patients who died at the ICU are already registered on *cause of death report*.)

6.4.2 Organ Function and Infection Reporting Status reporting for real time updating of database and for use in acute and planned interim analysis.
The following parameters are recorded continuously as an expression of vital organ functions and development of these (baseline = day 1 corresponds to the time of initiation of hypothermia treatment):
1. Cardiac / circulatory: Obtained discontinuation of vasopressor / inotropics on day 4 (a.m.6:00-9:00).
2. Respiratory: Achieved discontinuation of mechanical ventilation day 4 (a.m.6:00-9:00)
3.Renally: RIFLE "I". Has an increase in s-Creatinine at x 2.0 at any time from day 1 to day 4 (a.m.6:00-9:00) OR has a decrease in eGFR of > 50% (both endpoints)
4.Cerebrally: Positive CAM-ICU 72 hours after wakening up (no sedation).
5.Hepatically: Achieved Bilirubin> = 21 mM at 72 hours (Day 4)
6.Coagulation: Acquired decrease in platelet count> 25% day 1 to day 4
7. Infection: Achieved CRP decrease > 30% from day 1 to day 4
8. Days free of organ failure within 30 days: Organ failure is defined by: the need for mechanical ventilation, need for inotropic, RIFLE criteria, positive CAM-ICU.

This registration-sheet for organ function will be submitted on day 4 or first weekday after day 4.

At the coordination centre the data will be entered in the database within the first weekday after receipt. Delta factor (the increase rate) is calculated unless otherwise stated as:

$$\frac{\text{Day 4 value}}{\text{Day 1 value}} = \text{Delta Factor (e.g. D-CRP)}$$

8 separate O'Brien-Fleming plots are prepared.

6.4.3 Plan for the reporting of acute safety data for the Data Safety Monitoring Board.

The above mentioned data in section 6.4.2 are reported within 3 days after day 4 (~ 72 hours) and within 3 days after the patient has been discharged or died.

Once weekly the incoming data in the acute monitoring is up-dated, i.e.: 1) Primary endpoints, 2) Organ Function Reporting and Infection Status Reporting, 3) Calculation of causes of death with weights in the three categories cause of death 1) causes of death with emphasis on three causes of death categories: 1) not immediately related to the intervention, 2) possibly related to the intervention, 3) probably related to the intervention.

6.4.4 Acute reporting to the DSMB.

When crossing the safety limits of O'Brien-Fleming/Pocock plots for death (all-cause mortality-) or O'Brien-Fleming plots of Delta-factor (organ function), a brief report is made with the following three elements 1) death incl. documentation for exceeding the safety limits - data for the two groups compared, 2) determination of causes of death in both groups, 3) organ dysfunction in the two groups. Investigators are not informed about the safety report passed to the DSMB, in order to maintain equipoints and thus avoid trial damaging (and possibly safety compromising) behavior.

Extraordinary interim analysis: based on this DSMB may at any time require an extraordinary interim analysis and can require other data, besides the acute safety data, among the data recorded in the trial.

Investigators are only informed on extraordinary interim analysis if they lead to premature study closure or change in study procedures.

Adverse events will be listed for each group with maximum intensity, attributable to hypothermia and severity. Treatment-related adverse events leading to premature exit from the study will be calculated for both groups.

The following parameters will be presented in the planned interim analysis and on demand from DSMB, also by an extra ordinary interim analysis: Clinical biochemical and hematological blood test-results along with descriptive statistics. Changes from baseline levels in all these aspects will also be presented. Output levels are defined as the values measured on Day 1. Treatment-related toxicity will be categorized in relation to reference values for blood tests for the

two groups. A toxicity which can be seen in laboratory findings is considered treatment emergent if developed or increased in intensity after day 1 compared to the control group.

6.4.5 TABLE: Global overview of established and potential safety and interim analysis

Analyses	Description
Pause in the recruitment at 10 enrolled participants	After inclusion of the first 10 patients (minimum 5 in each group) recorded data are collected on: A) Primary endpoint B) Organ Function Reporting and Infection Status Report (see below, "planned IA) C) Extended coagulation (see below, "planners IA) Inclusion is paused temporarily and the DSMB will evaluate the effect of the intervention on participants in the group receiving hypothermia compared to the control group.
Pause in the recruitment at 50 enrolled participants	After inclusion of the first 50 patients (minimum 5 in each group) recorded data are collected on: A) Primary endpoint B) Organ Function Reporting and Infection Status Report (see below, "planned IA) C) Extended coagulation (see below, "planners IA) Inclusion is paused temporarily and the DSMB will evaluate the effect of the intervention on participants in the group receiving hypothermia compared to the control group.
Extraordinary interim analysis (Crossing the safety limits of O'Brien-Fleming plots)	Once weekly information in the acute safety monitoring is updated. These are: A) Primary endpoint B) Organ Function Reporting and Infection Status Report (see below "planned IA) C) Statement of reasons for death with the weighting of the three mortality categories (see Section 6.4.2) D) Incidence of severe bleeding: 1) CT confirmed ICH, 2) fresh upper or lower gastrointestinal bleeding 3) other surgery demanding form of bleeding. When crossing the safety limits of O'Brien-Fleming plots a report is send acute to the DSMB. Given the above mentioned information the DSMB may require an extra ordinary interim analysis including other collected and registered data.
Planned interim analysis (At inclusion of 100 - 250 - 400 participants)	The analyses are made by the DSMB and contains data concerning the primary endpoint, organ function reporting and

	infection status reporting, and any adverse event data before the current date. Primary Endpoint: Death of all causes within 30 days of enrollment Reports on organ function and infection status : 1. Cardiac: A) Inotropic score day 1, day 2, day 3, day 4 (time 0, 24, 48 and 72 hours) B) Inotropic score day 1+day 2 + day 3 + day 4 (time 0, 24, 48 and 72 hours) (accumulated need for inotropic) C) Achieved discontinuation of vasopressor/inotropic on day 2, day 3, day 4 (a.m. 4:00 to 9:00). 2. Respiratory: Achieved discontinuation of mechanical ventilation on day 3, day 4 (a.m. 4:00 to 9:00). 3.Renal failure: Increase in serum-Creatinine at x 2.0 at any time from day 1 = time of randomization before induction of hypothermia and day 4 (a.m. 4:00 to 9:00) OR decline in eGFR of> 50% (both providing endpoint). 4.Cerebral: CAM-ICU positive 24, 48 and 72 hours after awakening. 5.Hepatic: Delta Bilirubin. Analysis: Bilirubin (day 4, (a.m. 4:00 to 9:00) - Bilirubin (day 1) 6.Coagulation: A1) Delta platelets. Analysis: platelets (day 4, a.m. 4:00 to 9:00) – platelets (day 1) A2) Delta platelets/binary: Achieved decrease in platelets >25% day 1 → day 4. B) Delta INR. Analysis: INR (day 4, a.m. 4:00 to 9:00) – INR (day 1) C) Delta Factor II, VII, IX (”PP”). ”PP” (day 4, a.m. 4:00 to 9:00) – ”PP” (day 1). D) Delta APTT. APTT (day 4, a.m. 4:00 to 9:00) – APTT (day 1) E) Total consumption of SAG-M the first 10 days F) Occurrence of severe bleeding (1) CT confirmed ICH, 2) fresh upper or lower gastrointestinal bleeding 3) other bleeding with need for surgery).

	G) Trombelastrography (TEG) or Tromboelastrometry (ROTEM) is executed in the departments who have the opportunity. (Time R/CT (s)) Angel, MCF/MA. Day-to-day variability (unilateral variation analysis: ANOVA) Lysis30 min (lysis 30 min after maximum amplitude) 7.Infection: Achieved CRP decrease > 30% from day 1 to day 4. 8. Days without organ failure: Organ failure is defined by: the need for mechanical ventilation, need for inotropic, RIFLE criteria positive CAM-ICU.

7 UNEXPECTED INCIDENTS AND SERIOUS UNEXPECTED EVENTS

The primary and secondary endpoints and metrics listed above in the relevant sections, will be analyzed by interim analysis and included in safety analysis. As the intervention in the study (Mild Induced hypothermia) is a known, tested and accepted form of treatment, it is not relevant to apply the same procedures for this study as for studies where new drugs are tested.
However, investigators have a chance to report incidents which they find unexpected in a Case Report Form for unexpected events (CRF - "unexpected events"). In this form it is possible to classify the unexpected events related to antimicrobial therapy given as: "not related," "unlikely," "potential," "likely" or "related"

Serious unexpected events or unexpected events.

Serious adverse events and unexpected events which can be related to the appliquéd treatment of hypothermia should be reported immediately to the steering committee which is responsible to take the necessary actions.

The primary and secondary endpoints, which are recorded daily in the CRF are all *adverse* events and serious *adverse* events, such as death, complication of sepsis, increased antibiotic exposure and prolonged hospitalization. These are recorded routinely and daily in the general part of the CRF and not as an independent AE/SAE reporting in this group of patients who already at inclusion time is hospitalized due to life-threatening illness.

8 ETHICAL CONSIDERATIONS

8.1 Potential advantages and disadvantages

8.1.1 Potential benefits

Potential benefits are to improve the treatment of infection by inhibiting; the bacterial growth, the production of toxins by bacteria, the “programmed cell-destruction" and improve the body's ability to respond appropriately. This is described in detail in the background section.

Benefit to society: Based on the findings from this study, it will be possible with a high degree of certainty to conclude whether cooling in the form of mild induced hypothermia can lead to improved survival in "septic shock”, alternatively other benefits for patients with this diagnosis. If the study shows improved survival, it will have positive consequences for many patients with this diagnosis in the future.

8.1.2 Potential disadvantages

Potential disadvantages are the side effects that can possibly occur from cooling the body to 33 degrees Celsius. Under current indications for Mild induced hypothermia (mainly cardiac), documented side effects to treatment are primarily frostbite, where the cooling apparatus is in contact with the body.

To avoid these side effects as far as possible the patient will be closely monitored, so that the cooling apparatus can be moved to another area in the event of frostbite. The patient is sedated as long as chilling treatment is ongoing. All organ functions will be monitored (as is the procedure in intensive care patients in Denmark.) The specific monitoring is described in this section.

8.1.3 Reason for including unconscious patients

Mortality for patients with septic shock is at 40%-50% (4). Treatment of these patients in the ICU implies by default that the patient is intubated acutely upon arrival at the ICU (connected to a ventilator) and in connection therewith will be sedated to allow for further treatment and to address the high discomfort associated with being connected to a ventilator. Therefore, the patient will not be able to consent to participating in the CASS study. To make clinical research studies with the goal of improving the treatment of these life-threatening infections, it is therefore necessary to include unconscious and consciously blurred patients. It is not possible to conduct this study with another (awake) patient group and get the same result, as patients with infections, who do not have septic shock has a much lower mortality, and the pathogenic mechanisms of the disease, are very different for Septic shock patients and patients with infection, but without circulatory collapse. There will be obtained surrogate consent for patients who cannot decide on participation in the CASS study.

8.1.4 Reason that the study is conducted as an “acute study” in accordance with the Central Ethics Committee legislation § 20

Because septic shock is a condition which is acute life-threatening and where there is a great organ damage for every hour, it is extremely important to a given treatment it is initiated rapidly (within minutes to few hours) to give the patient the best chance of survival. The patient is not able to consent as the patient will either have weakened consciousness because of the acute illness, or the initial lifesaving treatment incl. sedation and intubation (addition of respirator) will make the patient acutely unconscious.
The acute lifesaving treatment cannot be deferred in order to ask the patient to decide on participation in CASS, as it would be a danger to the patient.

Expected improvement of the condition:
Conversely, bacterial infection is a reversible condition, and if the patient survives because of the treatment with hypothermia, it must be assumed the patient's health recover completely, so that he/she in most cases would get well, resume work/leisure and family functions.
Alternative treatments: There are currently no other treatments which have the ability to - before antibiotics have full effect in the focus of infection - reduce bacterial growth, reduce the bacteria's ability to produce toxins and to reduce the body's cells to combust in the period when the circulatory system fails.
This is a new treatment method that takes its background in the pathophysiological processes in life-threatening infections.

8.2 Regulatory Authority Approval

The project coordinator will (in collaboration with CASS coordination center) obtain approval from the relevant regulatory authorities prior to the start of the study in a department.
This study will be conducted in accordance with ICH-GCP and all applicable rules, including, where appropriate, Helsinki Declaration, June 1964, revised by the 59th WMA General Assembly, Seoul, in October 2008, see appendix 1.

8.2.1 Ethical approval

The study is approved by the ethical committees for the Capital Region (Region Hovedstaden).
It is the responsibility of “the investigator" to ensure that this protocol is evaluated and approved by the relevant local research ethics committee. The research ethics committee must also review and approve the informed consent forms used in the intensive care unit, and all other written information given to the patient prior to inclusion in the study. The project coordinator and / or "investigator" should send copies of the approval of the protocol and informed consent forms from the Ethics Committee to the CASS coordination center, and these copies must be received by CASS Coordination Centre prior to the start of the study.
If during the study it will be necessary to amend the Protocol or the informed consent forms, it is

the responsibility of the project coordinator and / or "investigator" that the local research ethics committee assesses and re-approve the amended documents. Approval from the local research ethics committee must be obtained before new patients can be included in the study under the amended form. Copies of the approval of the amended documents from the local research ethics committee must be sent from "investigator" to CASS coordination center as soon as they become available. The study will be recorded in the database for clinical trials www.clinicaltrials.gov and will be notified to the Data Inspectorate.

8.2.3 Legislation and Data Protection Agency

Information on the study-subject is protected under the Act on the processing of personal data and also under the Health Act. The project is reported to the Data Inspectorate.

8.2.4 Informed Consent

Patients are included acutely in the CASS study, due to the acute and life-threatening nature of the illness. Subsequent consent is urgently obtained from either the patient or alternatively a substitute informed consent will be obtained, see below as well as the specific instructions for these procedures.

"Investigator", or his / her delegated officer must then inform the patient about all aspects of patient participation in the study.

The process which leads to the achievement of this subsequent informed consent must be in compliance with all applicable regulations.

The patient alone (informed consent) or the patient and the substitute caregiver (substitute informed consent) will receive a copy of the signed and dated informed consent form and the original will be filed in the department's study archive. The decision made by the patient to continue his/ her participation in the study or alternatively not to continue, is completely voluntary.

"Investigator" or his/ her delegated officer must explain to the patient or his/ her lawful representative, that the consent to participate in the study may be withdrawn at any time without penalty or loss of benefits the patient would otherwise be entitled to.

If substitute informed consent is used, the patient must consent subsequently as soon as possible, please see appendix 4 +5 regarding this.

If the informed consent form is changed during the study, the "investigator" has to follow all applicable rules on obtaining approval from the local research ethics committee of the revised informed consent form and use of the modified form.

8.2.5 Quality assurance

On its own initiative, CASS coordination center perform a QA audit of this study. If such an audit is performed, "investigator" will accept to allow auditors access to all relevant documents and to be

available for the time needed - his/ her time as well as his/ her staff time - for auditors to conduct audits and discuss the relevant findings during audits. A guide for such an audit is available on the CASS coordination center

Furthermore, an external auditing body can perform an inspection of the study. If an external audit takes place, "investigator" will allow inspectors to access to all relevant documents and provide the time necessary as well as his/ her staff's time to the auditor to conduct inspection and discuss the relevant findings of the inspection .

8.2.6 Trial and Site Closure

By the end of study, the following activities will be performed, when possible, by the inspector (monitor) in collaboration with the "investigator" in an appropriate manner:

• archiving of all study data in CASS coordination center
• ensuring data unambiguity and clarity
• assessment of study department archives for perfection

CASS Steering Group reserves the right to temporarily or permanently discontinue the study at either a single study department or in all of these at any time and for any reason. If such action is undertaken selected members of the CASS Steering Group and / or CASS coordination center will inform and discuss this (including the reasons for the decision) with "investigator" at the study department. CASS coordination center will immediately inform all other "investigators" if the interruption of a study unit is based on a safety issue. "Investigator" must inform the local regulatory authorities as appropriate for the temporary or permanent interruption and the reasons for this. If required by the applicable rules, the "investigator" inform the local research ethics committee, and report on the cause of the interruption.

If the study is stopped prematurely all study data must be collected in CASS coordination center

8.2.7 Decentralized Filing

In accordance with the relevant rules, "the investigator" keeps a copy of data obtained at the study department after the study is completed locked in a safe place. CASS Coordination Center will inform the "investigator" on how long this archive is to be maintained to comply with the relevant rules. After this period, the archives can be closed properly.

8.2.8 Information confidentiality and secrecy

Confidentiality

"Investigator" and the study site personnel must keep all information related to this study, from the CASS coordination center (including this protocol) and all information generated during the study, confidential and must not use any information, data or files generated for any other purpose than

to perform the study. These restrictions do not apply to:
(1) information which becomes publicly available (e.g. publications) without error from the "investigator" or studio department personnel.
(2) Information necessary to submit in confidentiality to a local research ethics committee for assessment of the study:
(3) information necessary to send/ use in order to provide the proper treatment of a patient participating in the study.

8.2.9 Publications

The results from this study will be published in international “peer-reviewed "journals. If publication cannot be achieved in a relevant journal, the CASS Steering Group will ensure publication on a relevant website. CASS Steering Group decide if abstracts should be sent to conferences and how the results should be committed if more than one manuscript must be written. Results are published regardless of whether they are positive or negative regarding the main objective or one or more of the secondary objectives.

8.2.10 Authorship

The entire study group will appear in an appendix of all published manuscripts. Co-authors are selected for reasonable assessment of primarily the number of patients he/ she has contributed with and the level of involvement in the preparation of the manuscript. Provided that several manuscripts are made, a reasonable rotation of co-authorship will happen between the clinical study departments, though still considering the number of patients enrolled in the concerned study department.

8.2.11 Indemnify and Compensation for Damage

The insurance covering liabilities for patient treatment in Denmark, “Patient Insurance” will cover damage related matters, that may occur during performance of this study (48) (49).
Information on the subject is protected under the Act on processing of personal data and also under the Health Act. Datatilsynet (The Data Protection Agency) will be notified.

9 STUDY AND PROCEDURES

9.1 Case Report Forms (CRF)

Case Report Forms (CRF) will be provided by the CASS coordination center for each patient who participates. All data on the CRF will be entered legibly written in black ink or printed in Danish or English. Changes or errors in the CRF must not be erased or completely deleted, but a line must be put through the middle of the erroneous text and the corrected data added and dated by the

"investigator", a study approved colleague or study employee. An explanatory note regarding the change must also be added at the CRF. All information requested, but not obtained, or which cannot be answered must be identified by introducing an "ND" for (Not done). An explanation should be made for all missing data. The CRF must be updated regularly and should never bear patient's name or full Social Safety number (CPR). Patients would only be identified by initials, date of birth and study participant number.

"Investigator" (or a person assigned by the "investigator") must sign and date corrections on the CRF, where his/ her responsibility for the quality of the data is authenticated and where it also is certified that the data represent a complete and accurate record of each patient's participation in the study.

Details on the procedures for filling in the CRF are specified in the "*Manual of Operational Procedures*".

All study CRF's will be paper versions - the original will be "the investigator's" copy. After completion of each page of the CRF, the "investigator" sends it by. FAX to CASS coordination center. Pages will be compiled and the unambiguity in the data will be secured in accordance with the protocol-specific "Calculation and Validation Manual". Data will be entered twice (checked and verified) by separate data entering specialists to ensure quality of data files.

Identical validation checks will be performed on each database. Data which has not been checked will be marked and later come out as a separate file on a Data Clarification Report (DCR) and will be sent to the relevant "investigator" for consolidation. In such cases, "the investigator" will be asked to sign and date any explanation or correction. When the consolidated data is received, the databases will be updated accordingly and the original DCR will be filed along with the original CRF.

The database will be subjected to quality control (QC) before approval. Data will then be analyzed.

Evaluation	Day (screening & output value)		Day (counting from arrival at ITA) (follow up)				
	1	Daily routine	Day =discharge /death	30	60	90	180
Informed consent	X						
Inclusion criteria	X						
Demographics	X						
APACHE II/SOFA including. MAP	X	X	X				
Infections that occur during this hospitalization	X	X	X				
current diseases	X		X				
Health status	X						X
mortality		X	(X)	X	X	X	X
Inotropic	X	X	X		X	X	X
Hematology	X	X	X				
Clinical biochemistry	X	X	X				
Adverse events	X	X	X	X	X	X	X
Serious adverse events	X	X	X	X	X	X	X

Table 4: Clinical and laboratory evaluations

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11 APPENDIX

Appendix 1: The Helsinki Declaration , revised at the ”59th. World Medical Association General Assembly”, Seoul, and October 2008: To the investigators: This has been removed for space causes – can be required from the study coordinator or be downloaded from

http://www.wma.net/e/policy/pdf/17c.pdf

Appendix 2: Abbreviations and Glossary

ALAT	**Alanine Aminotransferase (SGOT)**
APACHE II	**Acute Physiology And Chronic Health Evaluation II**
ASAT	**Aspartate Aminotransferase (SGPT)**
CRF	**Case Report Form**
DDD	**Defined Daily Doses**
DIC	**Disseminated intravascular coagulation**
DSMB	**Data Safety and Monitoring Board**
”Investigator”	**Doctor responsible for compliance with the protocol**
ICU	**Intensive Care Unit**
IL-6	**Interleukine-6**
MODS	**Multi Organ Dysfunction Syndrome**
PASS	**Procalcitonin and Survival Study**
Protocol	**Project description /Detailed theoretical and practical description of the study**
Sepsis	**The presence of bacteria or their toxins in the blood**
Septic Shock	**Sepsis + drop in blood pressure**

APPENDIX 3: Guidance for initiation of hypothermia

The implemented form of Mild Induced Hypothermia at the local ICU, is used.

The appendix below applies to surface cooling with “body suit”/”cooling pads”.

The treatment with Mild Induced Hypothermia, regarding form (internal and external), begins with an infusion of isotonic NaCl 4ºC: 30 ml / kg

Cooling treatment - therapeutic hypothermia after cardiac arrest.

This section is borrowed from the intensive care unit at Hillerød Hospital, permission from senior doctor Morten Bestle, who is a part of the CASS project group.

Added to this section by the CASS project group is the section regarding *maintenance of normal temperature after 24 hours*.

NB: At all participating sites use approved equipment which is based on the principle "skin cooling" using cooling "pads" that are added to the patient's skin. Cooling is initiated with an infusion of cooled isotonic NaCl. Example of equipment: "Arctic Sun Energy Transfer Pads.

PROCEDURE

COOLING PHASE

Treatment goals: Core temperature at 33°C as soon as possible after return of spontaneous circulation (ROSC) after the collapse.

APPROACH

1. Infusion of isotonic NaCl 4°C: 30 ml/kg, 100 ml/min via 2 large peripheral iv. lines and by use of high pressure cuffs. Maximum infusion =3000 ml.
    a. Ex. 70 kg patient given 2100 ml NaCl at approx. 21 min.
    b. CAVE: Patients with chronic nephropathy should not get infusion with cold saline, but only cooled externally.
2. Priming of the hypothermia machine.
3. All clothes are removed from the patient and the pads are place. The patient must remain undressed without covering with sheets, diapers or anything else. For aesthetic reasons, the patient may be covered with a little blanket by visits.
4. If the hypothermia equipment is not available the patient is cooled using ice packs placed in the armpits, neck and groin. The bags should be wrapped in cloths to prevent frostbite. Additionally, a draw sheet soaked in iced water placed on the front of the patient.
5. Usual admission blood samples are taken.
6. Sedation and possible relaxation to reduce metabolism to a minimum. If the temperature is difficult to get down it may be due to shivering and hence muscle work which helps to keep the temperature up. Shivering is sometimes not visible, but should be suspected if tremor is increased/ elevated pCO2, rising tidalvolume or increased triggering of the respirator is observed.

7. Central venous catheter, arterial line, i.v- lines, feeding tube, bladder catheter with temperature sensor, and enhanced cardiovascular monitoring (LIDCO, CardioQ, PICCO or otherwise) should be placed as soon as possible. Due to increased bleeding risk these procedures should be made before the core temperature reaches 34 °C. The procedure should be minimally traumatic, especially if there is an indication for thrombolysis and anticoagulation treatment.

MAINTENANCE PHASE

Treatment goals: Core temperature is maintained at 33 °C for 24 hours (counted from the time the patient has achieved a core temperature of 33 °C).

Approach

Temperature

• The core temperature should be 33 °C for 24 hours starting from the time the temperature reaches 33 °C.

• If the patient experience shivering, it may be difficult to get the temperature down, since the muscle work in shivering helps to keep the temperature up. It may then be necessary that relaxes the patient. Shivering is sometimes not visible but is suspected by tremors, increased / elevated pCO2, rising tidalvolume or increased triggering the respirator.

Observe

The core temperature is recorded every 15 min. during cooling to 33 °C. Thereafter once every hour. If it is not possible to obtain more than one central temperature, the core temperature is recorded from the cooling machine.

Central Nervous System

Sedation and relaxation

During hypothermia medicine is dispensed according to the department's usual pain/sedation guidance.

Sedation: Inf. Propofol 0.1 to 0.5 mg/kg/hr in combination with

Inf. Remifentanil 0.05 to 0.2 ug/kg/min.

Muscle relaxation

• If using Remifentanil, when cooling is initiated, muscle relaxation is usually not necessary.

• Muscle relaxation by Shivering: inj. Esmeron 5 mg i.v . Can be repeated with 2-4 min. interval until end of Shivering.

• If Remifentanil is used, do not relax the patient, start with bolus inj. Esmeron. Bolus injection 0.6 mg/kg. The initial dose is only supplemented if shivering is later observed.

Observe: • Sedation; The patient must be heavily sedated, if necessary also relaxed.

• Pupils: checked regularly.

• Seizures and shivering

Circulatory

• MAP 65 to 100 mmHg sought

• CVP <20 cm H2O sought

• vasopressors - /inotropic treatment is carried out according to usual principles.

NB! The patient can develop bradycardia with a hemodynamic impact due to hypothermia.

Hemodynamic instability is treated according to the usual principles; either medical or using an external pacemaker (Zoll or a transvenuous pacing catheter). A slight increase in temperature to 34 °C can be accepted.

Respiratory

• Patient must be intubated.

• Controlled normal ventilation.

• Suctioning after regular instruction.

• Arterial puncture when needed

• **Hot water humidifier may not be used in the cooling period.**

• Provide inhalations with saline every 2 hours for the sake of humidification of the airways.

• CO2 monitoring

Observe: The usual observations for the intubated patient.

Gastro-intestinal

Nutrition

• Nasogastric feeding tubes must be placed before the temperature is <34 °C due to the bleeding risk.

• Attempts to start enteral nutrition up ASAP (prevents translocation)

• First day enteral nutrition is given with max. rate at 10 ml/hour. (Peristalsis is impaired by hypothermia and sedation).

• Elevated headboard, at least 30 degrees.

• Parenteral nutrition (including glucose) is not used.

Observe: Aspirate from the feeding tube in order to observe for gastric retention and bleeding according to guidelines as provided under the section of clinical supervision.

NB! Arrhythmia/cardiac irregularities where CPR may be needed, is a contraindication to tube feeding due to aspiration risk in cardiac massage.

Endocrinology

• Blood glucose levels are regulated according to usual guidelines.

• Blood glucose levels should be corrected before the temperature reaches <34 °C, as it then can be difficult to regulate blood sugar levels down and maintain it low.

Observe: Blood glucose levels are monitored frequently.

NB! There is often a need for Actrapid injections (refractory doses) without glucose.

Glucose infusion is not used for neurological unresolved patients, apart from hypoglycaemia event.

Renal

• Aiming TD> 1 ml / kg / hour

• Synthetic colloids should not be used due to bleeding risk.

• In declining diuresis is primarily given volume in the form of crystalloids, primarily isotonic NaCl or Ringer's Lactate.

NB! Hypothermia leads to reduced reabsorption in Renal distal tubules. Therefore, "cold diuresis" is expected. A positive fluid balance is likely the first day.

Paraclinical

• The core temperature is recorded every 15 min during cooling to 33 °C. Thereafter once every hour. If it is not possible with more than one central temperature, the recorded core temperature from the cooling machine can be used.

• Aim of potassium level is approx. 4 mmol/L. – It is likely to increase when rewarming due to the passes of potassium intracellularly during hypothermia.

• Magnesium, phosphate and ionized calcium remains within normal range.

Blood samples

• BS every hour

• ITA admissions tests on admission

• Se-Magnesium and Se-Phosphate should be checked 12 hours after cardiac arrest and in the morning

• DIC status in the morning and as needed.

• Arterial blood gas as needed

Medicine

• Antibiotics: The patient receives the first dose of antibiotics 1 hour after septic shock is diagnosed. Empiric treatment must meet the minimum criteria of piperacillin/tazobactam + ciprofloxacin or meropenem. If an abdominal focus and/or aspiration cannot be excluded metronidazole is also assigned. Of the drugs administered acutely, the most broad-spectrum is first provided (eg. If treated with meropenem + ciprofloxacin + metronidazole, start with meropenem then ciprofloxacin and metronidazole in the end).

• Ulcer prophylaxis with inj. Pantoprazole 40 mg x 1

• Thrombosis prophylaxis after the usual principle (Innohep or Fragmin).

Skin, tissue and care

• These patients are at high risk for pressure ulcers and should therefore be placed on a pressure relieving mattress. They should be closely observed for pressure from various wires. Relieve any pressure with micro-foam tape or other form of pressure-relieving material.

• Bedbath and other usual personal hygiene made to a limited extent, since there is a risk of heating of the

patient. Lower toilette is always preformed.

• Frequent foot and eye care is provided accordingly to guidelines and in relation to the sedated/relaxed patient. Wounds and injection sites are inspected and bandages changed according to guidelines.

• The patient can be turned and positioned at the side, provided that the circulation is stable and there is no elevated ICP. The patient must always have elevated headboard to at least 30 degrees.

• At any tampering with the relaxed patient, one should be aware that there is no muscle resistance. - Especially the neck must be supported eg by turning.

• Skin, mucous membranes, puncture sites, etc. observed for bleeding.

• Invasive entries; Central venous catheters, arterial lines etc. should preferably not be removed until the patient's temperature is below 34 °C due to increased risc of bleeding. If it is necessary to remove the object one should ensure the hemostasis and subsequent frequently monitor the site of injection.

• In case of x-ray the pads (Allon and Artic Sun) leaves a shadow on the image. One can open the part covering the exposed area.

• CT and MRI can be implemented with the pads, without causing artifacts.

HEATING PHASE

Approach

• The heating is started when the patient has been cooled for 24 hours, starting from the time the core temperature reaches 33 °C.

• Heating is slow, increasing core temperature with a maximum of 0.5 °C per hour.

• When the patient has regained normal body temperature, i.e. 36.5 °C, the bladder catheter is connected to monitoring equipment.

• The patient can be covered with a blanket when body temperature reaches normal temperature.

MAINTENANCE OF NORMAL THEMPERATURE AFTER 24 HOURS

Approach:

• In 72 hours from time of inclusion maintained normal temperature (36.5 °C -38 °C)

• 72 hours after time of inclusion removed cooling suit before the patient wakes up.

Special Features

Central Nervous System

• The patient should be sedated until normal temperature is reached i.e 37 °C core temperature and warm peripherally.

• Has muscle relaxants been used, discontinue this at 35 °C and used TOF monitor.

• If the TOF ratio is <0.9 and there are 3-4 scale of nerve stimulator, reversed, after ordination, with Robinul-neostigmine 0.04 mg / kg iv - Max. 5 mg neostigmine.

• If Remifentanil has been used, follow the procedure for withdrawal, see instructions for doing so.

• When rewarming begins, GCS scoring should be made every 4 hours.

Circulatory

• During rewarming peripheral dilatation occurs. Therefore infusion with fluids may be needed to maintain acceptable blood pressure.

Respiratory

• switch to hot water humidifier, when heating is started.

• The patient is extubated after the usual criteria.

Nutrition, glucose and potassium

• Nutrition conducted according to the usual principles.

• Blood sugar level checked frequently.

• Potassium should be checked frequently as potassium, during heating, passes extracellular and the large diuresis decreases.

Responsibilities and tasks

• The ITA physician prescribes the appropriate treatment and places the invasive catheters.

• The nurse launches and performs treatment in accordance to the present guidelines. Nursing staff observe and care for the patient as usual.

Relevant evidence in this area

1. Bernard SA, et al. Treatment of comatose survivors of our-of hospital cardiac arrest with induced hypothermia. NEJM 2002; 346:557-563.

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3. Nolan, JP et al. Therapeutic hypothermia after cardiac arrest. Resuscitation 2003; 57:231-5.

4. Rehn Power, A, Persson K, Bjørklund, P: Therapeutic hypothermia in IVA - new treatment collars specific kunskap. Valve, 2 / 2005, Sweden.

5. Intensivvård, Editors A. Larssson and Stone Rubertson, Liber A / B 2005

6. Hjärt-hjärneräddning after hjärtstopp, Omvårdnadsplan for hypothermia. Clinical guidance Anesthesia och intensivvård, IVA / Post-op, University Hospital of Lund, January 2005.

7. Horsted, TI, et al, Therapeutic hypothermia after cardiac arrest - a status Ugeskr. Physicians 2006; 168 (5): 458

Links: www.scctg.org