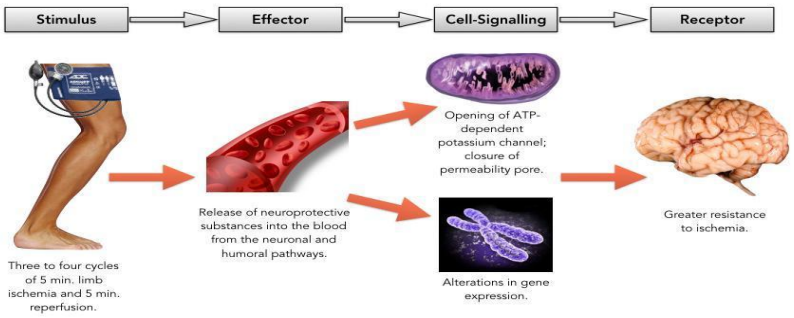
Clinical Investigation Plan

RESCUE-SAH trial



The effect of Remote Ischemic Conditioning on Delayed Cerebral Ischemia in patients with ruptured Aneurysmal Subarachnoid Hemorrhage: a randomized, patient-assessor blinded, sham-controlled study.

A prospective, randomized, patient-assessor blinded, sham controlled pilot study investigating whether remote ischemic conditioning (RIC) can improve clinical outcome in aneurysmal subarachnoid hemorrhage.

Project acronym	**RESCUE-SAH trial** The effect of **Re**mote I**s**chemic **C**onditioning on Delayed Cerebral Ischemia in patients with ruptured Aneurysmal S**u**barachnoid H**e**morrhage: a randomized, patient-assessor blinded, sham controlled study examining the effect on long-term clinical outcome
Clinical investigation plan	**Version 1.0, 5th of February 2023**
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Contents

1. Synopsis

Name of the principal investigator
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Title of study: RESCUE-SAH
The effect of **Re**mote I**s**chemic **C**onditioning on delayed cerebral ischemia in patients with r**u**ptured aneurysmal **s**ub**a**rachnoid **h**emorrhage.

Trial Management Groups (TMG):

Principal investigator/Sponsor (Aarhus University Hospital):
Grethe Andersen, Senior Consultant, MD, DMSc, Professor of Neurology, Department of Neurology, Aarhus University Hospital, DK-8200, Denmark.

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Trial Steering Committee (TMC):
Arzu Bilgin-Freiert (Aarhus University Hospital), Grethe Andersen (Aarhus University Hospital), Jens Christan H Sørensen (Aarhus University Hospital), Stig Dyrskog (Aarhus University Hospital), Ronni Mikkelsen (Aarhus University Hospital), Mahmoud Albarazzi (Aarhus University Hospital).

Data Monitoring Committee (and Trial Safety Committee):
The DMSC comprises of:
Independent neurosurgeon: Carsten Reidies Bjarkam, Senior Consultant, MD, PhD, DMSc, Professor of Neurosurgery, Aalborg University Hospital
Independent Neurologist: To be determined
Independent statistician: To be determined

Trial Endpoints Validation Committee:
EVC comprises of Independent senior consultants in Neurosurgery and Neurology

Trial monitoring:
During the entire study period quality assurance control will be performed.

Study centers:
Department of Neurosurgery, Aarhus University Hospital, DK-8200 Aarhus, Denmark

Planned study period: 2023-2033.

Phase of development:
Improve routine care of patients with aneurysmal subarachnoid hemorrhage and its secondary complications.

Objectives:

- To determine whether combined remote ischemic pre-, per- and postconditioning can improve clinical outcome in aneurysmal subarachnoid patients as an adjunct to standard treatment.

Diagnosis:

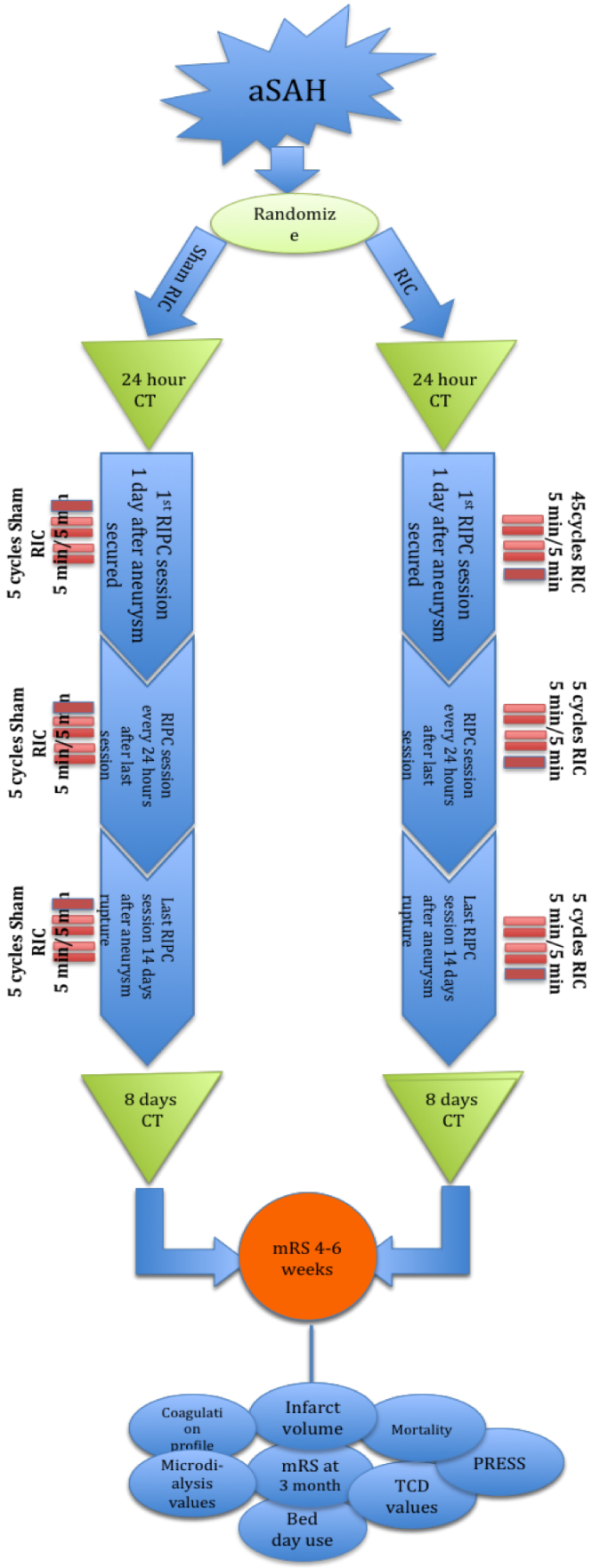
Aneurysmal Subarachnoid Hemorrhage (aSAH).
Methodology: An investigator-driven, prospective, randomized, pilot, parallel assignment, patient-assessor blinded, sham-controlled efficacy trial.
Randomization: ● Eligible patients will be randomized upon arrival at the hospital (via a secure web site). ● The patients and the clinical outcome assessor will be blinded to the treatment allocation.
Number and subjects (planned): ● 100 patients with full data sets
Inclusion criteria: ● Male and female patients (≥ 18 years) ● Aneurysmal subarachnoid hemorrhage confirmed by computed tomography (CT) with aneurysm origin confirmed by computed tomography angiography (CTA) or digital subtraction angiography (DSA) ● Aneurysmal subarachnoid hemorrhage symptom-onset ≤ 3 days ● Aneurysm protected by clipping or coiling ● Independent in daily living before symptom onset (mRS ≤ 2)
Exclusion criteria: ● Subarachnoid hemorrhage caused by a lesion other than cerebral aneurysm ● History of severe peripheral vascular disease or signs of severe peripheral vascular disease on physical examination ● History of deep vein thrombosis or signs of deep vein thrombosis on physical examination ● Pregnancy ● Concomitant other acute life-threatening medical or surgical condition
Criteria for evaluation: **Primary endpoints** ● **C**linical outcome after 6 months in patients with aSAH. Clinical outcome will be measured by modified Rankin Scale score. **Secondary endpoints** ● Difference in infarct volume in patient with aneurysmal subarachnoid hemorrhage (aSAH)

- Difference in occurrence of delayed cerebral ischemia in aSAH patients
- Early signs of microscopic vasospasm detectable by perfusion-ct scan, time-to-drain sequences.
- Occurrence of radiological signs of vasospasm at CT-cerebral angiography 7-8 days from ictus.
- Clinical outcome (mRS) after 3 and 12 months in patients with aSAH.
- Difference in TCD measures
- To determine continuous cerebrovascular autoregulation represent a predictive marker of secondary ischemia
- Proportion of RIC treated aSAH patients with cerebral vasospasm receiving inta-arterial drug infusion/therapy
- Three-month and one-year mortality aSAH and overall
- Early and very early neurological improvement in aSAH patients
- Overall Bed-day, and neurointensive care bed-day, use at 3 and 12 months and quality of life measures at 3 months
- To determine whether prestroke physical activity level (PASE) is a predictor for early and long-term recovery

Biochemical, blood and cerebrospinal fluid samples (tertiary and exploratory endpoints)

- Coagulation profile of aSAH patients in daily hospital obtained blood samples
- To determine whether RIC modulates coagulation in aSAH patients, and explore links between coagulation and infarct growth in aSAH and occurrence of DCI in aSAH
- Obtain a biobank of CSF from aSAH patients with an aim to explore potential prognostic CSF biomarkers with regards til DCI
- Obtain a biobank of blood samples from aSAH patients with an aim to explore potential prognostic CSF biomarkers with regards til DCI

Study flowchart


aSAH
Randomize
Sham RIC
RIC
24 hour CT
24 hour CT
1st RIPC session 1 day after aneurysm secured
RIPC session every 24 hours after last session
Last RIPC session 14 days after aneurysm rupture
1st RIPC session 1 day after aneurysm secured
RIPC session every 24 hours after last session
Last RIPC session 14 days after aneurysm rupture
5 cycles Sham RIC 5 min/5 min
5 cycles Sham RIC 5 min/5 min
5 cycles Sham RIC 5 min/5 min
45cycles RIC 5 min/5 min
5 cycles RIC 5 min/5 min
5 cycles RIC 5 min/5 min
8 days CT
8 days CT
mRS 4-6 weeks
Coagulation profile
Infarct volume
Mortality
Microdialysis values
mRS at 3 month
PRESS
TCD values
Bed day use


aSAH
Aneurism secured
Baseline Imaging 24 after aneurism secured
Randomise
5 cycles sham RIC (5min/5min)
Sham RIC
5 cycles RIC (5min/5min)
RIC
1st RIC/Sham RIC session Post hemorrhage day 2
Before and after RIC Bloodsamles and CSF collected TCD, ICP, PtO2 and microdialysis measures recorded
2nd RIC/Sham RIC session Post hemorrhage day 2
Before and after RIC Blood and CSF collected TCD, ICP, PtO2 and microdialysis measures recorded
3nd - 11st RIC/Sham RIC sessions Post hemorrhage days 4-13
Before and after each RIC Blood and CSF collected TCD, ICP, PtO2 and microdialysis measures recorded
Last RIC/Sham RIC session Post hemorrhage day 14
Before and after RIC Bloodsamles and CSF collected TCD, ICP, PtO2 and microdialysis measures recorded
Imaging after last RIC session
Follow Up
Mortality
Bed day use
mRS
Quality of life
Infarct growth
Early Neurological Improvement

Abbreviations

aSAH aneurysmal Subarachnoid Hemorrhage
CT Computed tomography
CTA Computed tomography angiography
DCI Delayed Cerebral Ischemia
DWI Diffusion-weighted imaging
DMC Data Monitoring Committee and Endpoints Validation Committee
FLAIR Fluid attenuated inversion recovery
END Early neurological deterioration
EVT Endovascular treatment
ICP Intracranial Pressure
MRI Magnetic resonance imaging
NPR Danish National Patient Register
PA Physical Activity
PreSS Prehospital Stroke Score
RIC Remote Ischemic Conditioning
RIPerC Remote Ischemic Perconditioning
RIPreC Remote Ischemic Preconditioning
RIPostC Remote Ischemic Postconditioning
TCD Transcranial Doppler

2. Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is one of the most devastating types of stroke. Half of the patients die during the acute ictus, and those who survive have a poor prognosis as 20-30% of them are disabled or eventually die from the disease.[1,2] In the acute phase after aSAH, the most devastating complication is rebleeding, whereas in the late phase, delayed cerebral ischemia (DCI) is another feared complication, associated with high morbidity and mortality[3–7]. Symptoms of DCI occur in 30 % of patients and 15-20 % of patients will develop a disabling stroke due to DCI. DCI typically occurs between days 4 and 14 after the initial bleeding. The cause of DCI is not fully understood. Cerebral vasospasms (CVS) are commonly seen on angiography in the first 4-14 days after initial bleeding, and have been linked to late onset of symptoms of focal ischaemia since the 60s. Modern research suggests that the pathophysiology is multifactorial, but CVS is still thought to be a major contributor.[8–10] Other contributing factors are thought to be microthrombosis, microvascular spasm, oxidative stress, cortically spreading depolarizations, cell death, breakdown of blood-brain barrier, among others[11,12]. Treatment with nimodipine is standard-of-care and is the only pharmacological intervention that has been shown to improve outcome in aSAH patients, although it has no impact on large-vessel CVS.[13] The need for developing effective methods for prevention or treatment of DCI persists, and an effective prophylactic treatment may have a large impact on the general outcome of aSAH.[14]

Ischemic conditioning is a potent activator of endogenous protection against ischemic injury. RIC can be applied as repeated short-lasting ischemia in a distant tissue that results in protection against subsequent long-lasting ischemic injury in the target organ.[15] This protection can be applied prior to or during a prolonged ischemic event as remote ischemic preconditioning (RIPreC) and perconditioning (RIPerC), respectively.[16] RIC is commonly achieved by inflation of a blood pressure cuff to induce 5-minute cycles of limb ischemia alternating with 5 minutes of reperfusion. RIC activates several protective mechanisms, through humoral and neural pathways and shows promise in the setting of acute stroke.[17,18]

Inflammation initiated by cerebral ischemia can contribute to secondary brain injury and is correlated with poor outcome. Following ischemia there is a harmful excess leukocyte infiltration in the brain parenchyma, and in experimental studies on aSAH, pharmacological inhibition of cytokines has been associated with improved outcome.[19,20] RIC has been demonstrated to reduce inflammation and downregulate inflammatory markers. In addition, RIC has protective effects on cerebral endothelial function and induces vasodilation, increasing cerebral blood flow (CBF).[21–32] Angiogenesis, erythropoietin and nitric oxide (NO) are suggested to induce neuroprotection and stimulation of these strategies by conditioning including inhibition of inflammation has the potential to play an important part in treatment of patients after aSAH.[33–36]
The effect of RIC on blood and cerebrospinal fluid biomarkers has never been explored in the setting of aSAH.

To-date, no serious adverse events have been documented in RIC. The procedure has been applied in numerous cardiovascular ischemic patients and in patients suffering from ischemic stroke and cerebral hemorrhage (ICH/SAH).[37–41] A recent smaller randomized trials of RIPreC after aSAH showed promising results with regards to functional outcomes and incidence of cerebral oxygen desaturation, likewise without adverse effects of RIC.[42–44]

RIC is a non-pharmacologic and non-invasive treatment without noticeable discomfort that has neuroprotective potential worldwide.[45]

Aneurysmal SAH and subsequent DCI represents a unique clinical opportunity to test RIC as DCI typically manifests within the first 14 days after ictus and is often a significant contributor to

neurological injury. The treatment is feasible, safe, and rooted in well-explored physiological concepts. There is a clear scientific gap and opportunity to explore RIC in the setting of aSAH and DCI in larger randomized trials.

3. Hypothesis

RIC may improve clinical outcome after 6 months in patients with aneurysmal subarachnoid hemorrhage, as measured by modified Rankin scale. We hypothesize that the patient group treated with RIC will have a lower incidence of radiologically verified vasospasm, DCI AND/OR less disabling lesions as a consequence of DCI, as compared to the group treated by sham-RIC.

Objectives

Primary endpoints

- Clinical outcome after 6 months in patients with aSAH. Clinical outcome will be measured by modified Rankin Scale score (mRS).

Secondary endpoints

- Difference in infarct volume in patient with aneurysmal subarachnoid hemorrhage (aSAH)
- Difference in occurrence of delayed cerebral ischemia in aSAH patients
- Early signs of microscopic vasospasm detectable by perfusion-ct scan, time-to-drain sequences.
- Occurrence of radiological signs of vasospasm at CT-cerebral angiography 7-8 days from ictus.
- Clinical outcome (mRS) after 3 and 12 months in patients with aSAH
- Difference in TCD measures between treatment arms
- Difference in measures of cerebrovascular autoregulation between treatment arms
- Proportion of RIC treated aSAH patients with cerebral vasospasm receiving inta-arterial drug infusion/therapy as compared to controls
- Three-month and one-year mortality aSAH and overall
- Early and very early neurological improvement in aSAH patients (Improvement from baseline GCS)
- Overall Bed-day, and neurointensive care bed-day, use at 3 and 12 months and quality of life measures at 3 months (WHO-5)
- To determine whether prestroke physical activity level (PASE) is a predictor for early and long-term recovery

Biochemical and cerebrospinal fluid (tertiary and exploratory endpoints)

- Coagulation profile of aSAH patients in daily hospital obtained blood samples
- To determine whether RIC modulates coagulation in aSAH patients, and explore links between coagulation and infarct growth in aSAH and occurrence of DCI in aSAH

- To explore potential CSF biomarkers for prognostication of DCI i aSAH.
- Obtain a biobank of CSF from aSAH patients with an aim to explore potential prognostic CSF biomarkers with regards til DCI

4. Trial design

Trial design

This is a prospective, randomized, patient-assessor blinded, sham-controlled pilot trial investigating whether RIC improves clinical outcome after 6 months in patients with aneurysmal subarachnoid hemorrhage.

Number of centers

Patients with aSAH from Aarhus University Hospital in Denmark will be recruited.

Number of subjects

We estimate that a sample size of 100 aSAH patients(full data sets) will be required to achieve sufficient data for evaluation of primary endpoint.

Sample size determination

Primary clinical endpoint

The treatment effect of RIC on long-term functional outcome and infarct size is unknown. The sample size calculation is based on the article from Van Donkelaar et al. (2019) describing the distribution of functional outcome according to modified Rankin Scale after 2 months[32]. Therefore we anticipate an effect size of a factor 0.6 on the long-term functional outcome with RIC compared to sham intervention.
The sample size is estimated with the formula:

$N=2 \times (Z^{1-\alpha/2} + Z^{\beta})^2 \times (SD/MIREDIF) \times (SD/MIREDIF)$

And with power at a alfa-level (significance) of 5% and power at a beta-level significance of 80 %,

N=2 x 16 x (1/0.6) x (1/0.6) = 90.

The sample size calculation was based on a simulation-based approach to the analysis of statistical power when logistic linear regression analysis is performed (significance level of 5%). To account for withdrawal and loss to follow-up we therefore plan to include 100 patients with full data sets. Lost to follow-up rate is estimated to be less than 5%. There is no planned replacement of patients lost to follow-up.

Randomization

Randomization procedure

The patient will be randomized to standard treatment with RIC or sham-RIC by the study coordinator at the receiving center. The randomization is based on a secure web site providing blocked randomization lists generated by a blinded, independent datamanager, stratified by the center. The investigator will make an assessment based on all available information whether the patient is eligible to participate in the study. Randomization is performed in- hospital.

Randomization will be stratified between Fisher grade 1-2 and Fisher Grade 3-4. Registered nurses or clinical assistants participating in the study will receive unique access and will have no influence on the randomization process.

5. Selection and withdrawal of subjects

Selection of patients

All patients with aSAH admitted to Aarhus University Hospital will be screened, and all who meet the study criteria will be included barring non-consent to participation.

- Depending on the randomization (RIC versus sham-RIC), patients with aSAH will continue RIC or sham-RIC treatment for an extended period after the aneurysm is secured.

Informed consent for enrollment

The inclusion:

In relation to enrollment, the patient's record will be reviewed to identify if the patient fulfills any exclusion criteria (listed below). Information on any patients excluded due to fulfillment of one or more exclusion criteria will be included for use in patient inclusion/exclusion flowchart (only the exclusion criterion will be registered).

The on-duty neurosurgeon/research physician will have examined the patient both physically and cognitively and will have assessed whether the patient is competent or not. ***The competent patient*** will be presented with a consent form with information about the study. The competent patient will receive oral information by the research physician based on the written patient information. The written patient information will be handed out (*deltager information*). We will ask for the patient's acceptance to be included in the study. The patient can withdraw consent at any time. In addition, the patient will have the usual requirements concerning a 24-hour time period for consideration and discussion with a lay representative. In-hospital inclusion and assessment of cognitive impairment will be undertaken during the admission at the neurosurgery center. Patients and next of kin will be given detailed information regarding the standard-of-care, and are informed that in the event of non-consent to inclusion, the patient will still receive standard-of-care treatment. Every effort will be made to inform the patients and relatives in quiet and undisturbed settings, this will be at the hospital bedroom or the designated room for conversations. We will prioritize the presence of nearest relatives at time of study information and inclusion.

The incidence of SAH is estimated at 9.1 per 100.000 per year. About 43 % of patients die from the initial bleeding and one third of those who survive the initial bleeding will die within 6 months if left untreated. In the acute phase, 20 % of aSAH patients may develop hydrocephalus. Patients with aSAH often because of the bleeding and development of hydrocephalus, experience an increase in the intracranial pressure causing confusion, cognitive impairment and/or loss of consciousness. The location and/or size of the aSAH, may also cause an altered mental status and some degree of cognitive impairment, rendering the patient incompetent[33]. Due to the nature of aSAH, we expect that a high percentage (around 80%) of patients will be incompetent at the time of inclusion, and the study detailed in this protocol cannot realistically reflect the patient population without inclusion of incompetent patients. The study entails minimal risk for included patients, but has the potential to greatly improve outcome for the individual patient. We submit that points 2a, 2b and 2c-iii of the NVKs checklist for inclusion of incompetent patients are fulfilled.

For ***the incompetent patients***, informed consent will be obtained from next of kin and an independent physician. The consenters will be provided information (based on the summary of the protocol for the independent physicians and study participant information for the relatives) on the trial to be able to make an informed decision about the patient's participation in this trial. The

independent physician will be the on-duty physician at the local department of neurosurgery, neurology, neuroanesthesiology, intensive care, or the on duty physician from another specialty with aSAH patient experience. On-duty physicians at these departments will receive detailed study information.

A patient who during the initial phase was legally incompetent and included as such, but during the follow-up period is assessed competent will receive oral and written information and we will ask for his/her informed consent.

Patients who fulfill the inclusion criteria will, according to randomization, receive RIC/sham-RIC treatment after the aneurysm is secured.

Once consent to participation is given, the investigators, sponsor and representatives of the sponsor, as well as any monitoring bodies, will have direct access to any information available in the patient's electronic journal. Access may be used to assess any details on the patient's prior health necessary for completion of the trial as well as for control purposes (including self-monitoring, quality control and monitoring of the study, all of which the TMG are obliged to perform).

Inclusion criteria

- Male and female patients (≥ 18 years)
- Aneurysmal subarachnoid hemorrhage confirmed by computed tomography (CT) with aneurysm origin confirmed by computed tomography angiography (CTA) or digital subtraction angiography (DSA)
- Aneurysmal subarachnoid hemorrhage symptom-onset ≤ 3 days
- Aneurysm protected by clipping or coiling
- Independent in daily living before symptom onset (mRS ≤ 2)

Exclusion criteria

- Subarachnoid hemorrhage caused by a lesion other than cerebral aneurysm
- Symptomatic vasospasm at the time of enrollment
- Previous cerebral lesion e.g. symptomatic cerebral infarction (>2cm), multiple sclerosis, symptomatic intracerebral hemorrhage, tumour, prior neurosurgery (excluding prior clipping or coiling of cold aneurysms without complications).
- History of severe peripheral vascular disease or signs of severe peripheral vascular disease on physical examination
- History of deep vein thrombosis or signs of deep vein thrombosis on physical examination
- Kidney involvement or prior kidney disease with an eGFR below safe levels for contrast infusion in relation to CT-perfusion.
- Pregnancy*

- Concomitant other acute life-threatening medical or surgical condition

**Women of child-bearing age should be asked about their use of safe birth control methods (contraceptive pill, intrauterine devices both hormonal and non-hormonal, hormonal implants, hormonal depot injection and transdermal hormonal patch). Women of child-bearing age will have s-HCG taken prior to final inclusion. If pregnancy cannot be ruled out,the patient can't be included. Women with a safe birth control method will be encouraged to use this method during the entire period of active RIC treatment.*

Method of blinding

Outcome assessment is blinded to the treatment leg and obtained by a clinical measure of the level of dependency and need for help in daily activities (**modified Rankin Scale**).
No information regarding randomization status will be recorded in the patient record.
Patients and the assessors of endpoints are blinded.

Discontinuation of study participation

A patient can withdraw from the study at any time. Patients can be withdrawn from the study at the principal investigator's discretion. In case the patient cannot be reached via telephone, every effort will be made to contact the patient or to document the outcome regarding new vascular events via registries/electronic health records. If the patient withdraws from the study, the date and the reason for the patient's withdrawal will be recorded. The patient is encouraged to provide information about his or her reason(s) for withdrawal and any experienced adverse effects (AE) during the study. Any patient who withdraws from the study will continue to receive standard in-hospital treatment and standard follow-up.

6. Study procedure and assessment

Identification of patient with aSAH

All patients with verified SAH on CT in the central Denmark region will be admitted to the neurosurgery center at Aarhus University Hospital and all patients with an aneurysmal SAH are identified by the on-duty neurosurgeon/vascular neurosurgeon, which is standard operating procedure. The aneurysm is then treated either by surgical clipping or endovascular coiling as standard operating procedure. All patients will be examined for neurological symptoms/findings and will have a structured assessment of Glascow coma score (GCS) before randomization.

Baseline data

Baseline data and process indicators are collected from the Danish Stroke Registry (DSR) and electronic health records. Additional information about medical history and medication and clinical and physiological data are collected at baseline. Data are recorded in an electronic case report form (e-CRF) at discharge from the Neurosurgery Center and on follow-up. For a complete list of registered data, see *Supplement B*

Brain autoregulation estimates:

The autoregulation impairment can be estimated by bedside computation of the correlation coefficient between ICP and mean arterial blood pressure (MAP). This produces a live estimate of how ICP reacts to changes in blood pressure. If the coefficient is zero or negative, it means that auto regulation is spared, and if positive, impaired.

This is an important difference distinguishing healthy brain tissue from tissue at risk of DCI due to aSAH, and hence autoregulation has been proposed to be a surrogate of brain tissue vitality, and could in turn represent a predictive marker of secondary ischemia after aSAH.

Magnetic Resonance Imaging (MRI) / Computed Tomography (CT)

Patients with aSAH diagnosis will have a CT perfusion with arterial angiography performed at Aarhus University Hospital Neurosurgery Center, 24 (16-32) hours after randomization as a baseline imaging. To differentiate the early and late infarction, the imaging will be performed again at 8 days after the initial bleeding. If issues should arise that present a contraindication to CT with intravenous contrast, an MRI will be performed in its stead wherever possible. If not, the patient will remain included for follow-up but will be excluded from the subcohort which had all planned radiography performed.

In case a patient develops clinical signs of DCI before the 8th day post-hemorrhage, the perfusion protocol will be added to the standard-of-care CT-STEALTH with angiography. This series of images will replace the day 8 imaging.

In case a patient develops clinical signs of DCI after the 8^{th} day post-haemorrhage, standard of care is to perform a CTC-STEALTH with angiography and perfusion. These imaging data will be included in the project.

Patients will have an MRI performed and in cases where MRI is contraindicated a CT will be performed, 4-6 weeks after randomization as a baseline imaging for the purpose of differentiating early and late infarction. The images will be analyzed by automatic or manual segmentation.

Ionizing radiation:

A CT-perfusion protocol has been set up at the department of Neuroradiology at Aarhus University Hospital We intend to supplement the standard-of-care protocol, which includes CT-STEALTH and a CT Cerebral angiography performed after surgery to ensure sufficient closure of aneurysm.

The perfusion protocol has been tested on anthropomorphic phantoms intended for estimating CT-dose indices ($CTDI_{vol}$). $CTDI_{vol}$ multiplied by scan length in cm produces the *Dose-Length Product* (DLP), which may be expressed in mSv by multiplying by a set conversion factor, for CTC, the factor is 0.0018.

The CT-perfusion protocol has a DLP of 1569.8, the STEALTH-protocol has a DLP of 776.5, and the angiography has a DLP of 229.0.

Thus, the total estimated DLP of the protocols intended for use in this study is

$DLP_{study =} (DLP_{CTperfusion} \times 2) + DLP_{angiograpy} + DLP_{STEALTH} = (1569.9 \times 2) + 776.5 + 229 = 4145.3$ mGy x cm

Or, expressed in mSv

4145.3 mGy x cm / 0.0018 mSv/mGy x cm = 7.46 mSv

Therefore, with 1 planned CT-perfusion protocol added to the post-operative standard of care CT+CTA-protocols, and another scan (CT STEALTH, angiography and perfusion) at the 8th day after initial bleeding the total dose of study-related radiation should remain below 10 mSv during the course of the study.

It is important to note that patients with aSAH will have CT-STEALTH and CT-angiography performed as standard-of-care (2 at minimum, baseline and control after aneurysm has been secured).

The total exposure to radiation in this protocol falls under category IIb of NVKs appendix 2, as the category covers 0-10 mSv of exposure. It should be noted that the experimental treatment has the potential to prevent an often devastating complication of aSAH, and that CT protocols including perfusion and angiography imaging is the gold standard for monitoring development of DCI in patients during intensive care, which in turn provides invaluable information about efficacy of treatment in the present study.

Most patients diagnosed with aSAH in Denmark are around 50 yrs of age.

Blood samples

Patients with aSAH diagnosis will have baseline blood-samples drawn 24 (16-32) hours after randomization. Blood samples will be drawn again before and after RIC on day 2, 4, 7 and 10. These samples will supplement the standard operating procedure. 40mL in total will be drawn at the time of each sampling. If the patient develops signs of DCI, further samples are drawn on the day the patient develops these, and the following day, regardless of timeframe. No more than 80 mL in total will be drawn in a day.

Blood samples will be stored until completion of the trial in a research biobank.

A research biobank will be established, and all blood samples obtained during the study are stored here. During the entire study and analysis period, the material will be stored at Aarhus University Hospital. The last patient will be included in 31. May 2027. Analysis of blood samples, according to secondary endpoints, will be performed during the study and until 6 years after inclusion of the last patient (no later than 31. May 2033). Hereafter the remaining material will be transferred to a biobank for future unspecified research purposes. The study will be registered in the internal registry of scientific projects in the central Denmark Region, for this additional 20-year storage period of the blood samples (until 31 May 2053), after that, the samples will be destroyed. All analysis will be performed in Denmark.

Cerebrospinal Fluid samples

There are many potential neurological and medical complications following aSAH. Acute temporary unconsciousness after aSAH is very common due to high intracranial pressure caused by either the initial hemorrhage, hydrocephalus or both, even in patients with minor aSAH. Increases in ICP from hydrocephalus are associated with secondary brain ischemia, therefore prompt recognition and aggressive treatment is necessary[34]. Patients with aSAH developing hydrocephalus will be

treated surgically by placing an intraventricular catheter for external drainage of cerebrospinal fluid as a standard operating procedure**.** The CSF production is approximately 500 mL pr. day and there is normally equilibrium between its production and absorption. Disruption of the equilibrium may cause buildup of fluid and cause intracranial hypertension. The insertion of external drainage as standard of care reduces risk of symptomatic intracranial hypertension by draining excess cerebrospinalfluid[35].

Patients with aSAH diagnosis who have an external drainage of cerebrospinal fluid as a standard operating procedure will have cerebrospinal-samples taken from the external drainage pre-and post RIC after randomization. This will be repeated on day 2, 4, 7 and 10. 20 mL will be drawn daily until 14 days after surgery. This is not standard operating procedure, but poses no risk to the patient since there is need for drainage because of excess fluid. Even in patients without raised ICP, 20 mL is an inconsequential amount due to the daily production, which normally exceeds 500 mL. As the samples are taken from the external drainage, the 20 mL are taken from what is already excess fluid, drained as standard operating procedure.

Cerebrospinal fluid samples will be stored in a research biobank until completion of the trial.

A research biobank will be established, and all cerebrospinal fluid samples obtained during the study are stored here. During the entire study and analysis period, the material will be stored at Aarhus University Hospital. The last patient will be included by May 31 2027. Analysis of cerebrospinal fluid samples, according to secondary endpoints, will be performed during the study and until 6 years after inclusion of the last patient (no later than 31 May 2033). Hereafter the remaining material will be transferred to a biobank for future unspecified research purposes. The study will be registered in the internal registry of scientific projects in the central Denmark Region, for this additional 20-year storage period of the cerebrospinal fluid samples (until 31 May 2053), after that, the samples will be destroyed. All analysis will be performed in Denmark.

ICP and blood pressure monitoring

Patients with intraventricular drainage or ICP monitoring, used as a clinical tool at Aarhus University Hospital Neurosurgery Center, will be monitored for ICP changes before, during and after each RIPC session after randomization. ICP changes in the event of DCI will also be recorded.

Patients with an Arterial cannula will be monitored for changes in blood pressure in relation to RIPC as well as in relation to developing DCI.

ICP and blood pressure are continuously monitored using ICBM+ Software (Cambridge Enterprise Ltd, University of Cambridge, Cambridge, UK).

Transcranial Doppler Monitoring

Transcranial Doppler (TCD) non-invasively measures CBF velocities and cerebrovascular hemodynamics within the basal arteries of the brain. During TCD, ultrasound is transmitted through thin areas of the skull, e.g. windows. These sound waves reflect off blood cells moving within the blood vessels, allowing for the calculation of their speed based on the Doppler effect[36]. As a standard operating procedure at Aarhus University Hospital Neurosurgery Center, TCD recordings will be performed daily in all patients with aSAH from the day of admission until day of discharge.

TCD monitoring will be performed in patients with aSAH before, and after each RIC session after randomization.

Brain Microdialysis/Brain Tissue Oxygen Monitoring

In patients with aSAH, cerebral microdialysis or Raumedic Neurovent-PTO can be used as a clinical tool to monitor brain metabolism and/or brain tissue oxygen monitoring during neurointensive care as a standard operating procedure. After randomization the brain metabolic changes and brain tissue oxygen monitoring will be monitored before, during and after the RIC sessions. After RIC sessions microdialysis samples will be collected continuously every hour as a clinical tool to monitor brain metabolism as standard operating procedure. Glucose, lactate, Lactate/pyruvate ratio, glycerol and pyruvate will be measured immediately.

The Raumedic Neurovent-PTO system has the advantage of brain tissue oxygen monitoring (PbtO). In patients with heightened risk of cerebral ischemia such as patients with aSAH the Raumedic Neurovent-PTO system can detect hypoxia when it occurs. The normal value for PbtO in the brain ranges from 25 to 50 mmHg. Values below 15 mmHg indicate cerebral ischemia, and values below 5 mmHg indicate cell death. After randomization, the Raumedic Neurovent-PTO values will be registered before, during and after the RIPC session in patients with a Raumedic Neurovent-PTO inserted as a standard operating procedure.

Remote ischemic conditioning protocols

Device

RIC will be performed using an Automatic Tourniquet System 2200 (A.T.S. 2200), produced by Zimmer Biomet. The A.T.S. 2200 is an automated pneumatic tourniquet device, developed for safely regulating the interruption of blood flow during operations. It regulates the pressure of a tourniquet cuff, which temporarily occludes blood flow to a patient's upper or lower extremity to maintain an anemic field. The device is designed to safely maintain interruption of blood flow to an extremity for up to 120 minutes, and has built-in alarms that serve to ensure that time thresholds are respected.

The device is classified as class I according to annex VIII of the MDR under EU law, produced by the US-based company Zimmer Biomet, Warsaw, Indiana, USA. In Denmark, the Device is imported, sold and serviced under , and marketed in Denmark and maintained by Zimmer Biomet Denmark ApS, Herstedlund 12, 2620 Albertslund, Denmark. Both companies are CE, QM, MDSAP and ISO 13485 certified.

Using the Zimmer A.T.S. 2200 for RIC falls under the intended functions covered by the CE-marking of the device. The device has previously been approved for use in Danish randomized trials, regarding interventions in orthopedic surgery.

Remote Ischemic Conditioning (RIC)

After randomization a large thigh blood pressure cuff will be placed on the lower limb. The cuff will be inflated to a pressure 30 mm Hg greater than the systolic arterial blood pressure measured by the patient's arterial line or upper limb blood pressure cuff. The adequate level of inflation will be

confirmed by the absence of pulse in the ipsilateral pedal artery detected by palpation. If the pulse signal is still present, the cuff will be inflated further until it disappears. The cuff will remain inflated for 5 minutes. Then the cuff will be deflated and the limb allowed to re-perfuse for 5 minutes. The procedure will be repeated five times followed by reperfusion. The first session will be performed 24-72 hours after the initial hemorrhage, within the first 24 hours after treatment of aneurysm, and repeated every 24 hours, between 8-10 am, until post hemorrhagic day 14.

Sham – remote ischemic conditioning (Sham-RIC)

After randomization a large leg blood pressure cuff will be placed on the lower limb. The cuff will be inflated to a pressure 20 mm Hg. The cuff will remain inflated for 5 minutes, then the cuff will be deflated for 5 minutes. The procedure will be repeated for five cycles. The first session will be performed 48-72 hours after the initial hemorrhage, at least 24 hours after treatment of aneurysm and repeated every 24 hours, between 8-10 am, until post hemorrhagic day 14.

RIC/sham-RIC treatment will continue for a total of 14 days. If patients are discharged from the hospital, treatment will be halted upon discharge.

RIC/sham-RIC protocol	**Accepted time frame**
First RIC	Within 24 hours after aneurysm secured
Second RIC	23 hours from last cuff of the initial RIC
RIC daily until post hemorrhagic day 14	23 hours from last cuff of the last RIC until day 14

Table 2: Schedule and Activities

Days after treatment (First RIC the morning after surgery)	ICTUS/ 0	1	2	3	4	5-6	7	8	9	10	11-14	Discharge	Wk 4-6	90 days	180 days
Informed Consent		X													
Medical history	X														
Medication	X														
Weight	X														
Physical examination	X														
Baseline imaging	X														
Surgical intervention	X														
Clinical evaluation, standard-of-care		X	X	X	X	X	X	X	X	X	X	X			
RIC/ sham-RIC		X	X	X	X	X	X	X	X	X	X				
Blood sample before and 1-2 hours after RIC*			X		X		X			X					
CSF sample Before and after RIC*			X		X		X			X					
TCD		X	X	X	X	X	X	X	X	X	X				
CTC, perfusion, angiography*		X						X							
MRI-c													X		
Follow-up interview														X	X
mRS-score												X		X	X

7. Endpoints

An independent end-point committee will adjudicate clinical events.

Primary endpoints

Criteria for evaluation

Clinical outcome at 6 months in patients with aSAH

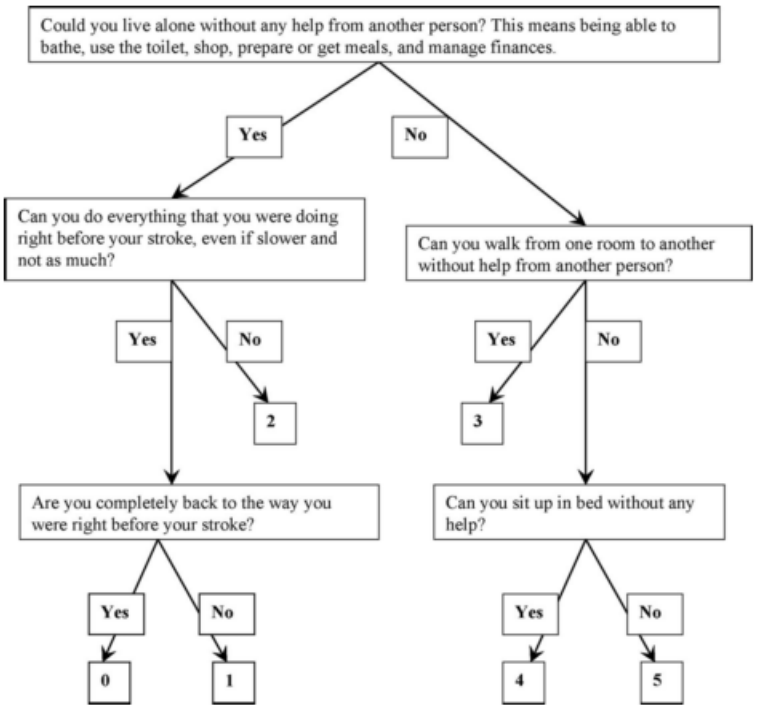
The level of dependency and need for help in daily activities will be determined by either face-to-face assessment or based on structured telephone interviews performed by assessors who are blinded to the intervention at 3 and 6 months after the index aSAH.

The assessment will be performed by two independent blinded assessors, which can be either face-to-face or telephone based assessment (not same day), and the patient will be contacted by a third assessor (face-to-face or telephone) who is blinded to the intervention who will assess the level of dependency.

- If disagreement occurs between **two telephone assessments** – a third, and final, telephone or face-to-face assessment will be made.
- If disagreement occurs between one face-to-face assessment and one telephone assessment – the face-to-face will be considered the final assessment
- If disagreement occurs between **two face-to-face assessments** – a third, and final, telephone or face-to-face assessment will be made.

Every possible effort will be made to assess the outcome in patients who are unable to participate in the interview (this could be due to post-stroke neurological impairment (for example aphasia and/or dysarthria), or that the patient cannot be reached by telephone). The outcome will then be assessed by contact to a named relative or the general practitioner. Several attempts to contact the patient by telephone will be made before contacting the named relative or general practitioner. Death in the follow-up period will be obtained from the Danish Civil Registration System (CPR).

The structured telephone interview will be based upon a validated Danish translation of the “slightly revised simplified, modified Rankin Scale questionnaire”[37]. The translation and its validation will be performed according to the AAOS guideline[37,38].



Bruno et al. 2011. Stroke.

Furthermore, modified Rankin Scale assessors are required to have received specific mRS training, which could be an online mRS certification: https://secure.trainingcampus.net/uas/modules/trees/windex.aspx?rx=rankin-english.trainingcampus.net

Outcome	Accepted time frame (from symptom onset)
3, 6 and 12-month follow-up – 1st and 2nd assessment	3, 6 and 12 months +/- 2 weeks
3, 6 and 12 month follow-up – 3rd assessment	3, 6 and 12 months and 2 weeks +/- 2 weeks

Secondary endpoints

Clinical outcome attributed to secondary cerebral ischemia in aSAH

Early neurological improvement in aSAH patients

Neurological deficits and daily neurological assessment are documented with the GCS.

- **Early neurological improvement** is defined as*:*

Increase in GCS from baseline or resolution of symptoms at discharge.

- **Increase in GCS ≥ 2 points or resolution of symptoms after 24 hours in subgroups:** endovascular treated aSAH.

Diagnosis of aSAH and the clinical outcome is documented in the electronic case report form. The daily difference in neurological impairment during the hospital stay will be documented in all randomized patients. Neurological deficits are documented using Glasgow Coma score (GCS). The GCS score is obtained and registered by the on-call neurosurgeon before randomization. Glasgow Coma Score is assessed at 24-hour by a certified and trained general neurosurgery nurse/neurosurgery research nurse/physician/neurosurgeon. Only information regarding time and place of assessment, patient name, age and social security number will be available. The GCS score of patients with aSAH and non-aSAH diagnoses, are performed by the on-call physician/neurosurgeon or a research nurse/general neurosurgery nurse. *mRS is documented at discharge*. The outcome assessor will be alerted in advance of the assessment. The mRS is recorded in the electronic CRF.

Secondary infarct attributed to secondary cerebral ischemia in aSAH

Secondary infarct growth is defined as the difference in infarct volume between baseline and 14 days on CT. In all aSAH patients, the baseline imaging modality is CT. In cases of initial CT, a baseline with CT perfusion is performed 24-hours after the aneurysm is secured. To estimate the infarct volume the imaging will be performed at day 8 after the initial hemorrhage and 24 (16-32) hours after the last RIC/sham-RIC session or before discharge. Patients will have an MRI performed 4-6 weeks after randomization to differentiate the late infarction.

Imaging protocol	**Accepted time frame**
CT perfusion baseline – aSAH	24 hours after aneurysm secured
CT perfusion (8 days)	8 days from initial bleeding
MRI (4-6 weeks)	4-6 weeks from initial bleeding

Information about imaging protocols, see *Supplement A*

Ischemic vascular events at 3 and 12 months in aSAH patients

Ischemic vascular events defined as:

- Acute ischemic stroke
- Transient ischemic attack
- Myocardial infarction, ST-elevation myocardial infarction (STEMI), and Non-STEMI

Three-month and one-year mortality

Information about mortality is collected using the CPR and LPR, at two time points (6 and 15 months after the inclusion of the last patient). All-cause mortality is assessed and subdivided into vascular mortality versus non-vascular mortality[39].

Analyses are performed on all randomized patients and according to subgroups.

Endovascular treatment in RIC treated aSAH patients with cerebral vasospasm

There is a rapid advance in the endovascular treatment of cerebral vasospasm following aSAH and the primary goal is to increase CBF to prevent infarction. The common modalities of endovascular vasodilatation are an intra-arterial drug infusion and/or balloon angioplasty[40].

Difference in proportion of RIC treated aSAH patients with cerebral vasospasm receiving intra-arterial drug infusion/therapy.

Quality of life measures at 3, 6 and 12 months in aSAH patients

. Quality of life and bed-day use measures in aSAH patients

Quality-of-life measurements are assessed by telephone interview at 3, 6 and 12 months after inclusion. The modified Rankin scale and WHO-5 questionnaire are used. Reliability to determine the patient's mRS category score is satisfactory both when assessed by telephone interview and by consultation[46], and the WHO-5 is validated for use in telephone interviews[47]. Baseline WHO-5 is obtained at Aarhus University Hospital.

Link to: Danish National Board of Health - WHO-5.
Information about bed-day use via the LPR and the local stroke registry.

Biochemical profile of aSAH subtypes and of RIC-induced neuroprotection

Biochemical substudies

- Coagulation, fibrinolysis, endothelial and inflammatory marker profile in obtained blood samples to differentiate between baseline infarct, secondary infarct, and non-infarct.
- Microvesicular changes in relation to aSAH
- MicroRNA changes in relation to aSAH
- Modulation of the mentioned biomarkers by RIC in aSAH.

Handling of blood samples:

Blood samples will be collected from patients with aSAH. After randomization the blood samples will be obtained through arterial cannula from patients in the ICU, or via standard peripheral venous blood sampling where not possible. The local biochemical department or study personnel will centrifuge the blood and divide the plasma into cryotubes that will be kept at 80 °C for long-term storage.

Patients with aSAH diagnosis will have baseline blood-samples drawn 24 (16-32) hours after randomization. Blood samples will be drawn again before and after RIC on day 2, 4, 7 and 10. This is not standard operating procedure. 40mL in total will be drawn at the time of each sampling. If the patient develops signs of DCI, further samples are drawn on the day the patient develops these, and the following day. No more than 80 mL in total will be drawn in a day.

All blood samples obtained during the study will be stored in a research biobank

Blood-samples, in the research biobank, will be stored until completion of the trial (no later than 31. May 2033). Hereafter the remaining material will be transferred to a biobank for future unspecified research purposes (see section “Blood Samples” under “study procedure and assessment”).

Planned biochemical analysis

Coagulation assays:
Functional and immunologic plasma assays will be employed to analyze proteins and pathways in coagulation, fibrinolysis, endothelium and inflammation. The analyses will be performed in the accredited clinical laboratory and the thrombosis and hemostasis research unit at the Department of Clinical Biochemistry, Aarhus University Hospital. Plasma samples will be stored in cryotubes at -80 °C until analysis is undertaken in large batches.

Overview, blood samples:

Analyses	Vials	Volume (ml)	Handling	Place of analysis
Routine coagulation analysis				
aPTT, INR, fibrinogen, fibrin d-dimer, antithrombin	Blue Na-citrat, 1 x 1,8 ml	1,8	Delivery in the 24-7 lab within 1 hour of sampling	24-7 lab, dept of clinical biochemistry
Biobank				
Secondary hemostasis: TG, F1+2, TAT Fibrinolysis: clot-lysis, PAI-1 Endothelium: sTM, VWF, P-selectin Biobank for future research	Blue Na-citrat, 2 x 9 ml	18	Centrifugation and aliquoting, storage at -80°C until analysis	Research lab, dept of clinical biochemistry
Neurofilament light chain and other neurological biomarkers Biobank for future research	Serum 1 x 9 ml	9	-	-
Markers of complement activation Proinflammatory cytokines Biobank for future research	Purple K-EDTA 1 x 9 ml	9	-	-
Total (max)		**37,8 ml**		

aPTT: activated partial thromboplastin time; INR, international normalized ratio; TG: ex vivo tissue factor induced thrombin generation; F1+2: prothrombin fragment 1+2; TAT: thrombin-antithrombin Complex; clot-lysis: ex vivo tissue factor induced fibrin generation or -lysis; PAI-1, plasminogen activator-inhibitor 1; sTM, soluble thrombomodulin; VWF, von Willebrand-factor

Handling of Cerebrospinal samples:

Patients with aSAH diagnosis who have an external drainage of cerebrospinal fluid as a standard operating procedure will have cerebrospinal-samples drawn pre- and post RIC on day 2, 4, 7 and 10 after randomization. Samples will be taken from the external drainage. This is not standard operating procedure. 10mL will be drawn at all times.

Brain microdialysis:

In select aSAH patients where cerebral microdialysis (CMA 70, microdialysis AB, Stockholm, Sweden) is being used as a standard operating procedure to monitor brain metabolism during neurointensive care, the brain metabolic changes will be monitored before, during and after the RIC sessions with a standard flow rate of 0.3 µL/min, which leads to a higher recovery. After randomization we will collect the baseline (pre-session) vial measures the hour before the start of the RIC and the second vial will be collected during RIC sessions (the inflation–deflation rounds), which represents the average ischemic effect of the five inflations. After RIC sessions samples will be collected continuously as a clinical tool to monitor brain metabolism. Glucose, lactate, L/P ratio, glycerol and pyruvate will be measured immediately (CMA 600 microdialysis AB, Solna, Sweden).

Brain Tissue Oxygen Monitoring

In select patients with aSAH, Raumedic Neurovent-PTO can be used as a standard operating procedure to monitor brain tissue oxygen monitoring during neurointensive care. The Raumedic Neurovent-PTO system can give an early warning of a significant difference between the cerebral tissue oxygen supply and oxygen demand. After randomization the brain tissue oxygen monitoring will be recorded. All values will be registered including before, during and after the RIC sessions.

Physical activity (PA) measures in aSAH patients

Patients and their relatives will be asked to complete the physical activity in the elderly (PASE) questionnaire during the acute hospital admission. In patients who are unable to complete it themselves an structured interview based on the PASE questionnaire will be performed[41,42].
The PASE is a 12-item questionnaire, which quantifies the amount of PA over a 7-day period. The PASE questionnaire was developed with the purpose of assessing the level of PA in middle-aged and elderly individuals. The PASE score is calculated by taking the average number of hours spent on an activity (sports, occupational activity, household activities, and leisure time activities) per day over a 7-day period multiplied by an activity coefficient. Item scores are added to reveal the PASE score. The PASE score may range from zero to more than 400.

8. Benefits of the study

Potential benefits:

Participating patients with aSAH may experience an improvement of clinical outcome and reduction in secondary cerebral ischemia.

Disadvantages:

Mild-to-moderate pain and petechiae in the RIC-treated leg may occur during the inflation of the blood pressure cuff. It should be noted that all patients are on a standard-of-care regime of analgesics following aSAH and surgical intervention, which should mediate any pain. Otherwise, the RIC treatment has been tested in large populations of patients with ischemic stroke, intracerebral hemorrhage as well as in large populations of patients with cardiac ischemia, and has so far proven safe and without serious side effects. Recent smaller trials have tested RIC in patients with aSAH, also without any serious adverse events.

Sham-RIC will only be associated with a slight sensation of pressure on the lower extremity.

Baseline CT and CT angiography are obtained for primary assessment (according to hospital SOP). An additional (compared to standard operating procedure) head CT-perfusion will be performed. The CT scan will be repeated during the patients hospital stay (according to hospital SOP) in aSAH patients and an additional (compared to standard operating procedure) head CT-perfusion protocol will be performed at day 8 if the patient's clinical status allows. CT-scans are associated with a dose of radiation. The CT-protocols associated with the study will not exceed 10 mSv (see "Ionizing Radiation" above). This corresponds to about 3 years of background radiation in Denmark.

An additional head MRI will be performed 4-6 weeks after the initial aSAH bleeding. In cases where MRI is contraindicated we will perform a head CT perfusion. The MRI scan is harmless and not associated with radiation risk. The scan may be associated with discomfort due to noise and claustrophobia.

9. Assessment of safety

Emergency unblinding procedure

In cases where emergency unblinding is necessary the on-call neurosurgeon will log-on to electronic *CRF at redcap.dk and enter* the civil registration number (CPR) of the patient. The treatment can now be unblinded. All changes will leave an audit-trail.

Adverse events

Patients are monitored in the neurosurgery unit with GCS scoring at close intervals, and neurological examination when indicated, and adverse events are treated according to clinical guidelines.

The Data Monitoring Committee will assess safety according to primary study endpoints, rebleeding, recurrent stroke, myocardial infarction and mortality.

Interim analysis of safety parameters in aSAH will be performed regularly during the study.

During the hospital stay the patients will be asked if they have experienced any deterioration of health or new symptoms during or after RIC. Furthermore all patients will have a 24/7 contact number to the neurosurgery center and a contact to study research physician and will be instructed to report any deterioration of health, development of new symptoms or other adverse events.

All possible serious adverse events (SAEs) will be registered in the e-CRF and stratified by seriousness (benign, serious, serious unexpected). All cases of re-bleeding from a secured aneurysm will be reported as possible adverse events for evaluation. All possible adverse events will be examined for causality by an independent evaluator. Petechiae in the treated extremity as well as moderate pain are expected and mild adverse events, and will not be reported.

Suspected unexpected serious adverse reactions /events (SUSARs) will be unblinded and reported directly to the committee of research ethics. SUSARs deemed lethal or life-threatening will be reported to the sponsor immediately upon discovery. The sponsor will inform the committee of research ethics within seven days of the SUSAR.

All discharged patients (and relatives) will be given contact details to study personnel and be instructed to report any deterioration of health or new symptoms. The patients with aneurysmal subarachnoid hemorrhage will once again be asked for adverse events at the 3- month telephone interview. In this group, end-of-study visit is the last telephone interview, whereas end-of-study visit for participants without a vascular diagnosis is at the time of discharge from the neurosurgery center.

All patients who have consented are followed (through Danish Health Registries, LPR and DSR) for new vascular events and mortality for up to 12 months.

Event report and causality assessment

All events will be registered in the electronic CRF and reported to the authorities at the interim analysis. A yearly safety report, containing a list of all SAEs will be submitted to the Independent Ethics Committee by the Sponsor as required by Danish law.

10. Project timetable and recruitment feasibility

Study preparation: January 2022 to May 2023

Study start date: June 2023

Month 0-3: Patient recruitment

Expected inclusion end date: 31. May 2027

Month 2-5: Study analysis

Expected end date for last analysis and follow-up: 31. May 2033

Annual admissions of aSAH patients at the neurosurgery department at Aarhus University Hospital is approximately 70 patients. Approximately 40% of those patients die due to acute ictus or rebleeding before surgery can be initiated. It is estimated that we will include about 30-35 patients annually.

11. Ethical considerations

The study will await approval from the Regional Ethical Committee. The study will comply with the Data Protection Act and data protection regulation. Enrollment of additional Danish stroke centers will require a new application to the regional ethics committee (amendment).

Any discomfort and stressing of the patients can potentially put patients at risk of rebleeding and therefore we will randomize the patients after the aneurysm is secured. Improvement of standard care in patients suffering from aSAH is of paramount importance. Inclusion of all aSAH patients is necessary in order to translate any positive research results into a benefit for all aSAH patients. The intervention is without any known risk apart from moderate pain in the lower extremity when the cuff is inflated, and has been explored in the setting of intracerebral hemorrhage, ischemic stroke and cardiac ischemia previously without reported serious adverse events[18,45] (REF).

There is a risk of infection during cerebrospinal sample draw, but to minimize this risk samples will be drawn by experienced personnel under sterile, secure conditions. Patients frequently have CSF drawn daily for analysis, and samples can be taken concurrently to minimize any additional risk.

Study participants are covered in accordance with the Danish Patient Insurance Act.

12. Data handling and record keeping

All study data are recorded in an electronic CRF with blinded data and identification via a study identification number. The study will apply to the specifications of the act on processing of personal data. The study will also comply with the Data Protection Act and data protection regulation.

Baseline data and process indicators are collected from the Danish Stroke Registry (DSR) and electronic health records. Additional information about medical history and medication and clinical, paraclinical and physiological data are collected by the examining physician at baseline, and taken from the electronic health records upon inclusion. Data are recorded in an electronic case report form (eCRF) during the admission period, at discharge from the Neurosurgery Center and at follow-up. For a list of registered data, see *Supplement B*.

Study data were collected and managed using REDCap electronic data capture tools hosted at *Aarhus University Hospital*.[48,49] REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

Patient participation will be recorded in the medical record. Data will be stored at the Department of Neurosurgery, Aarhus University Hospital, for 15 years, after which the documents will be shredded.

A notification to The Danish Data Protection agency will be submitted.

The investigator permits direct access to all source data/documents (including electronic patient record) at monitoring visits, audits and/or inspections by the regional ethics committee.

The informed consent permits the investigators, sponsors and its representative and the control authority direct access to all source data/documents from patient records and electronic records to keep information about the study participant's health information that can necessitate completion of the project, control purposes such as self-control, quality control and monitoring which are required to perform.

13. Publications policy

The results of the study negative, inconclusive and positive will be disseminated as widely as possible - through publication in an international peer-reviewed journal, as conference presentations and on www.clinicaltrials.gov.

The trial will be registered on www.clinicaltrials.gov and www.strokecenter.org/trials.

After the trial period, a report on the device efficacy as a mediator of RIC, as well as a shorter resumé will be published online and referred to in the various publications as well as on clinicaltrials.gov.

14. Statistics

Primary study endpoint:

Difference in clinical outcome (mRS) at 6 months in aneurysmal SAH (General ordinal logistic regression analysis. Significance level of 5%)

Ordinal logistic regression analysis, expressed as a common odds-ratio[50]. This method is encouraged by the European Stroke Organization's Outcomes Working Group, both for calculating sample sizes and analyzing results of clinical trials.

Secondary study endpoints (selected):

- Difference in infarct growth after 4-6 weeks (Binomial regression analysis, significance level of 5%)
- Difference in modified Rankin scale during the first 14 days: (general ordinal logistic regression analysis, Significance level of 5%)
- Difference in early neurological improvement in aSAH (general ordinal logistic regression analysis. Significance level of 5%)
- Difference in occurrence of delayed cerebral ischemia in aSAH patients (ordinal logistic regression analysis, significance level of 5%)
- Three-month and one-year mortality (Two sample test of proportion (Chi-square test), significance level of 5%)
- Difference in the coagulation profile (general ordinal logistic regression analysis, Significance level of 5%)
- Difference in presence of vasospasm at cerebral angiography baseline vs 8-day scan (two-sample test of proportion (chi-square test), significance level of 5%)

Interim analysis

We will perform an interim analysis for evaluation of the actual event rate after inclusion of 50% of the patients. If this shows a lower infarct size than expected, a new sample size calculation will be performed.

The trial can be stopped at interim analyses for futility, efficacy and safety reasons.

Missing data:

Missing data will be handled using multiple imputation (predictive mean matching imputation).

15. Source data access and monitoring

Trial-related audits and/or monitoring will be provided by direct access to source data/documents. The sponsor will conduct a yearly audit and compile a report of any adverse events.

16. Economy

Study initiators are Grethe Andersen, Senior Consultant, MD, DMSc, Professor of Neurology at Department of Neurology, Aarhus University Hospital and Jens Christian H Sørensen, Senior Consultant MD, PhD, DMSc, Professor of Neurosurgery, Aarhus University Hospital.

The Danish foundation TrygFonden has supported the study with 3.695.172 dkk (research personnel
salaries, running costs and imaging).

The Danish Foundation Region Midtjyllands Sundhedsvidenskabelige Forskningsfond has supported the study with 898.396 dkr (study investigator/coordinator salary).

The full amount of granted funds flows through the Department of FAS (Regnskab, Forskning og Eksterne midler), Aarhus University Hospital which will then approve and manage dispersal including all project-related expenses and remuneration, in accordance with the project budget.

The funders have no role in study design, data collection, analysis or interpretation, nor the decision to
publish or the preparation, review and approval of any part of the manuscript.

Supplements

Supplement A - Neuroimaging protocol

On admission CT is performed according to hospital SOP.

All aSAH patients can be included in RIC/sham-RIC based on a baseline native CT. However, after the aneurysm is secured there is a risk of infarct either complications to the procedure or from the initial hemorrhage and therefore patients with aSAH after randomization require a CT perfusion at baseline demonstrating acute ischemic lesion in order to evaluate infarct growth. Baseline head CT perfusion in aSAH patients is performed within 24 hours after the aneurysm is secured and before the first RIC series.

Non aSAH diagnosis (only evaluated with CT) will not be included in the RIC study and no further neuroimaging outside the standard care will be performed.

Imaging protocol	**Accepted time frame**
CT perfusion baseline	24 hours after aneurysm secured
CT baseline	24 hours after aneurysm secured
Control CT perfusion	8 days from baseline CT
MRI	4-6 weeks from baseline CT

Computed tomography (CT) protocol
Baseline CT and CT angiography are obtained in centers using CT for primary assessment (according to hospital SOP).

CT analysis

Infarct volume assessment at baseline (24 hours after aneurysm is secured), and at 8 days will be performed by an experienced neuroradiologist. Any visible vasospasm at angiography will also be registered. The assessment will be assisted using an automated CT Neuro perfusion workflow software for stroke volume assessment (e.g. Siemens, SyngoVia, Aarhus University Hospital, Denmark)[43].

Magnetic resonance imaging (MRI) protocol
Diffusion-weighted imaging (DWI), apparent diffusion coefficient (ADC), T2* gradient-recalled echo (T2*GRE), and T2 fluid-attenuated inversion recovery (T2-FLAIR).

The protocol for 4-6 weeks MRI includes: DWI, T2*, T2 and T2-FLAIR.

The total acquisition time for this protocol is approximately 10-20 mins (MRI), depending on the MRI equipment.

MRI analysis

Follow-up MRI, 4 -6 weeks after the initial bleeding, the DWI and FLAIR lesion will be outlined, representing irreversibly damaged tissue.

Infarct volume assessment at 4-6 weeks is performed by an experienced neuroradiologist. The assessment may be assisted using an automated stroke volume assessment software.

Supplement B – Baseline data

Age	Prior or active long-course immunosuppressive treatment
Male/female	Prior or current chemo or radiation therapy in relation to head/neck areas.
Medical History	Prior or current phosphodiesterase-5-inhibitor treatment
Hypertension	Prior or current stimulant drug use (amphetamine, ephedrine, cocaine)
Smoking	
Alcohol	**Clinical and physiological data**
Hyperlipidemia	Symptoms on admission and worsening of symptoms, if any.
Diabetes	Modified Rankin Scale prior to aSAH
Previous myocardial infarction *Yes: within 3 months, yes: more than 3 months, no, unknown*	Symptom onset (time)
Angina pectoris	Time of admission
Atrial fibrillation	Completed prehospital RIC/sham cycles
Previous ischemic stroke or TIA	= 5, 4, 3, 1-2, 0
Peripheral artery disease	Time (start/stop) for each RIC/sham cycle
Physical activity (PASE interview) Substudy at Aarhus University Hospital	Admission blood pressure
Medication on admission	DCI onset
Statins	Stroke onset
Calcium channel blockers	Modified Rankin Scale (t = 14 days)
ACE inhibitors/angiotensin receptor blockers	**Endovascular spasmolysis therapy**
Beta-blockers	Treatment initiation (time)
Platelet inhibitor treatment	**Paraclinical data**
Anticoagulation therapy	Diagnostic CT-scan /MRI made on admission for assessing severity of aSAH (Fisher scale)
Direct oral anticoagulation treatment (DOAC)	**Mode of securing aneurysm**
Opioid treatment	Coiling or Clipping
SSRI treatment	

Baseline data obtained from treating physicians, database of Danish stroke centers (ddsc.dk), Danish stroke registry and electronic health records.

Supplement C – Study Design

All patients with verified SAH on CT will be admitted to the neurosurgery department at Aarhus University Hospital. All patients with an aneurysmal SAH are identified by the on-duty neurosurgeon/vascular neurosurgeon, which is standard operating procedure in Denmark. After the aneurysm is secured, the research physician will make an assessment based on all available information whether the patient is eligible to participate in the study. If the patient is eligible, the patient or family members will be informed by the study coordinator/registered nurse, on-duty neurosurgeon/vascular neurosurgeon or a neurocritical care physician and signed consent will be obtained. The patient will be randomized to standard treatment with RIC or sham-RIC by the study coordinator at the receiving center. Registered nurses participating in the study will receive unique access and will have no influence on the randomization process.

Prior to the first round of RIC, the aneurysm has to be secured by clipping or coiling. Patients will have a CT perfusion performed 24 (16-32) hours after the aneurysm is secured as a baseline imaging to confirm the early infarction that can occur due to the initial aneurysm rupture or as a result after the treatment from clipping or coiling of the aneurysm.

The neurocritical and neurosurgery team decides if there is an indication to place a brain microdialysis catheter, Raumedic Neurovent-PTO catheter, external drainage or ICP monitor. Once it is indicated the same team will place it. The hemodynamic and ICP changes will be monitored during the RIC procedure. Once all monitoring is in place the study team will perform the RIC procedure.

As a standard procedure TCD measurements will be obtained from the day of admission to the patient's hospital discharge and once the patient gives consent for the study, TCD will also be measured during and after the RIC procedure. Similarly blood and CSF samples will be taken during and after the RIC procedure.

RIC will be induced by inducing Ischemia in the lower extremity. In the neurointensive care unit, the arms often have numerous monitoring devices and catheters inserted, which make their use for conditioning problematic. The RIC sessions will consist of 5-minute cycles of lower limb ischemia followed by 5-minute periods of reperfusion. The procedure will be repeated five times followed by reperfusion. The aim is to start the RIC procedure at post hemorrhage day 2 and perform RIC sessions every 24 hours between 8-10 am until post hemorrhage day 14. The RIC procedure will be performed via an adult large lower extremity blood pressure cuff wrapped around the thigh. The cuff will be inflated to a pressure 30 mm Hg greater than the systolic arterial blood pressure as measured by the patient's arterial line or upper limb blood pressure cuff. The adequate level of inflation will be confirmed by the absence of pulse in the ipsilateral pedal artery .The cuff will remain inflated for 5 minutes. The cuff will then be deflated and the limb will re-perfuse for 5 minutes, after which the procedure will be repeated. In sham-RIC, the cuff will be inflated to a pressure 20 mm Hg. The cuff will remain inflated for 5 minutes then the cuff will be deflated for 5 minutes. The procedure will be repeated for five cycles daily.

The device used for inflating the cuff is the Zimmer A.T.S. 2200, a CE-marked device intended for creating a bloodless working environment during surgeries of a duration up to 2 hrs.

Head CT perfusion imaging will be performed 24 hours after securing the aneurysm and on day 8 post-hemorrhage. To differentiate late infarction, an MRI will be performed 4-6 weeks after the initial hemorrhage.

Patients with aSAH diagnosis who have an external drainage of cerebrospinal fluid as a standard operating procedure will have cerebrospinal-samples drawn pre- and post RIC on day 2, 4, 7 and 10 after randomization. This is not standard operating procedure. 10mL will be drawn at all times.

Patients with aSAH diagnosis will have baseline blood-samples drawn 24 (16-32) hours after randomization. Blood samples will be drawn again before and after RIC on day 2, 4, 7 and 10. This is not standard operating procedure. 40mL in total will be drawn at the time of each sampling. If the patient develops signs of DCI, further samples are drawn on the day the patient develops these, and the following day. No more than 80 mL in total will be drawn in a day.

The primary end point is patient modified Rankin Scale score at 6 months. MRS will be evaluated at admission (prior mRS), discharge, at 3 months, 6 months and 12 months.

Supplement D – Roles and Responsibilities

Name	Role	Responsibility
Grethe Andersen, MD, DMSc, Professor of Neurology	Sponsor, Principal investigator	Principal investigator
Jens Christian Sørensen MD, DMSc, Professor of Neurosurgery	Professor, department of Neurosurgery	Coordination, inter-department logistics
Arzu Bilgin-Freiert, MD, PhD, Associate Professor	Study Coordinator	Investigator
Stig Dyrskog, MD	Neuro ICU Coordinator	TCD measures
Mads Rasmussen, MD	Neuroanesthesiologist	Patient transport to Imaging
Leif Østergaard MD, Professor Ronni Mikkelsen MD	Neuroradiologist	Image description and measurements
Søren Paaske Johnsen, Professor	Statistician	Data analysis

Claus Vinter Bødker Hviid	Department of clinical biochemistry, Research division	Coordination of (exploratory) biomarker studies, CSF
Julie Brogaard Larsen	Department of clinical biochemistry, Thrombosis and Hemostasis, Research division	Coordination of (exploratory) biomarker studies, coagulation
Kim Morgenstjerne Ørskov	PhD Student, investigator	Investigator, Coordinator

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