Remote Ischemic Preconditioning in Head and Neck Cancer Reconstruction - A Randomized Controlled Trial

Aim
The aim of the trial is to investigate if remote ischemic preconditioning improves the outcome of microsurgical reconstruction.

Literature review
Overview of systematic literature review performed in PubMed/MEDLINE:

| Search terms (April 7, 2015)                                                                 | Identified references: |
|---------------------------------------------------------------------------------------------|------------------------|
| "Ischemic Preconditioning"[Mesh] AND "Hemostasis"[Mesh]                                     | 20                     |
| "Blood Coagulation"[Mesh] AND "Ischemic Preconditioning"[Mesh]                              | 4                      |
| "Ischemic Preconditioning"[Mesh] AND "Fibrinolysis"[Mesh]                                  | 2                      |
| "Thrombosis"[Mesh] AND "Ischemic Preconditioning"[Mesh]                                    | 20                     |
| ischemic preconditioning coagulation                                                       | 35                     |
| "Surgical Flaps"[Mesh] AND "Ischemic Preconditioning"[Mesh]                               | 78                     |
| "Surgical Flaps"[Mesh] AND "Ischemic Preconditioning"[Mesh] AND "Hemostasis"[Mesh]        | 0                      |
| "Surgical Flaps"[Mesh] AND "Ischemic Preconditioning"[Mesh] AND "Thrombosis"[Mesh]       | 0                      |

Additional literature was identified through review of reference lists from relevant publications.

Background
In microsurgical reconstruction, the patient’s own tissue is applied for reconstruction of defects following tumor resection or trauma. Blocks of tissue are harvested from a donor site and transferred to the defect, where the blood supply is restored by microvascular anastomoses under the operating microscope. These tissue blocks are termed ‘free flaps’. For example, the fibula bone with a skin paddle from the lower leg is applied for reconstruction of the jawbone and oral cavity following resection of oral cancer.

Thrombosis to the vascular anastomoses and microcirculatory intravascular thrombosis are the main reasons for total flap necrosis. Total flap necrosis occurs in 1-9 per cent of cases with devastating consequences for the patient [1-3]. Therefore, research aimed at reducing the risk of thrombosis in free flap reconstruction is essential.

The surgical procedure results in a hypercoagulable state, endothelial dysfunction, and altered blood flow in the patient’s circulation during free flap reconstruction. It is well known, that oncologic patients are in a hypercoagulable state with an increased risk of thrombosis [4]. Ischemia-reperfusion injury to the flap, and the suture line of the anastomoses, are the main reasons for endothelial dysfunction in the flap. Furthermore, stasis and hypoxia in the patient’s circulation contribute to endothelial dysfunction. These changes in the coagulation system result in an increased risk of thrombosis and have major impact on the outcome of free flap reconstruction.
Ischemic preconditioning, where brief periods of ischemia and reperfusion are induced prior to prolonged ischemia, has been shown to attenuate ischemia-reperfusion injury of organs subject to acute ischemia [5]. Ischemic preconditioning can either be applied locally on the organ prior to ischemia, or anatomically remote by ischemic preconditioning of tissue separate from the target organ, termed remote ischemic preconditioning [6]. Furthermore, remote ischemic perconditioning, applied during target organ ischemia, and remote ischemic postconditioning, applied at reperfusion of the ischemic organ, have been described as methods to reduce ischemia-reperfusion injury [7-8]. Randomized controlled trials with remote ischemic perconditioning as an adjunct to revascularization in patients with myocardial infarction or ischemic stroke have shown promising results [9-10].

The mechanism behind remote ischemic conditioning is unknown, but the research has focused on a humoral response or a neurogenic response [11-13]. Furthermore, effects involving the immune system and coagulation system have been investigated [14-16]. Ischemic preconditioning and remote ischemic preconditioning of experimental free flaps have been shown to increase flap survival and attenuate ischemia-reperfusion injury [13,17-20]. However, just one clinical case series with six preconditioned abdominal flaps for breast reconstruction has been published. And two clinical studies with healthy subjects have shown, that remote ischemic conditioning improves the cutaneous microcirculation at a free flap donor site [21-23]. Hence, no randomized controlled trials have been conducted to show the efficacy of remote ischemic conditioning in free flap reconstruction.

Research team
This randomized controlled trial is performed at the Department of Plastic Surgery and Center for Hemophilia and Thrombosis, Department of Clinical Biochemistry at Aarhus University Hospital (AUH), Aarhus, Denmark. The trial is part of a Ph.D. project at the Graduate School of Health at Aarhus University. The research team consists of:

- Study chair and sponsor: Professor Anne-Mette Hvas, M.D., Ph.D., Center for Hemophilia and Thrombosis, Department of Clinical Biochemistry, AUH.
- M.D./Ph.D.-student: Andreas Engel Krag, Center for Hemophilia and Thrombosis, Department of Clinical Biochemistry, AUH.
- Birgitte Jul Kiil, M.D., Department of Plastic Surgery, AUH.
- Christine Lodberg Hvas, M.D., Ph.D., Department of Anesthesiology, AUH.

Study aims
1) To investigate if remote ischemic preconditioning attenuates platelet aggregation in head and neck cancer patients undergoing microsurgical reconstruction.
2) To investigate if remote ischemic preconditioning induces fibrinolysis in head and neck cancer patients undergoing microsurgical reconstruction.

Hypotheses
1) Remote ischemic preconditioning attenuates platelet aggregation.
2) Remote ischemic preconditioning increases fibrinolysis.
**Study participants, methods and materials**

**Design**: Randomized controlled trial.

**Study intervention**: Included patients will be randomized in the operating room after induction of general anesthesia to remote ischemic preconditioning or no remote ischemic preconditioning (sham). Patients are blinded to the study intervention. Remote ischemic preconditioning will be carried out by upper extremity ischemia using an inflatable tourniquet. Four cycles of 5 min ischemia followed by 5 min of reperfusion will be administered. In patients randomized to sham, the tourniquet will be applied to the patient, but not inflated.

**Eligibility**:
Inclusion criteria:
- At least 18 years of age.
- Ability to give informed consent.
- Will undergo resection of a malignant tumor in the head and neck region immediately followed by microsurgical reconstruction with a single free flap at the Department of Plastic Surgery, AUH.

Exclusion criteria: Arterial or venous thromboembolism in the previous three months.

**Outcomes**:
Biochemical data:
- Primary hemostasis: Platelet count and platelet aggregation (Multiplate®).
- Secondary hemostasis: aPTT, INR, antithrombin, and thrombin-antithrombin complex.
- Fibrinolysis: Fibrin clot lysis, fibrin d-dimer, prothrombin fragment 1+2, plasminogen activator inhibitor-1, tissue-plasminogen activator, plasminogen, and thrombin-activatable fibrinolysis inhibitor.
- Global hemostasis: Thrombin generation.
- Systemic inflammation: C-reactive protein, leukocytes, fibrinogen, interleukin-6, and von Willebrand factor (antigen).

Blood samples will be drawn at:
1. Just before induction of general anesthesia.
2. At randomization (intraoperatively).
3. 3 h after RIPC/sham.
4. 6 h after RIPC/sham.
5. 06:30 AM on the 1st postoperative day.

Clinical data:
- Previous treatment for head and neck cancer
- Co-morbidities
- Medication
- Course of hospitalization
- Re-operations
- Morbidity and mortality

Follow-up for clinical data is 30 days postoperatively. Data will be collected from review of patients’ electronic medical records and postoperative visits to the Plastic Surgery Clinic, AUH.
Obtaining informed consent
Potential study participants will be encountered in the multidisciplinary Head and Neck Cancer Clinic, AUH, where newly referred head and neck cancer patients are evaluated for surgery by head and neck surgeons, plastic surgeons, maxillofacial surgeons, and oncologists. Patients who are scheduled for surgery, and who meet the inclusion criteria, will receive verbal and written information about the clinical trial from Dr. Birgitte Jul Kiil, Dr. Gete Toft Eschen, Dr. Hans Henrik Nielsen, or M.D./Ph.D. student Andreas Engel Krag. This conservation will take place in a private clinic room with no interruptions, and the patient’s companions are encouraged to join the conversation. Informed consent for trial participation will either be obtained immediately, or the patient is offered time for deliberation until the day of admission before surgery. Under normal circumstances the patient will be scheduled for surgery within 14 days from their first visit to the Head and Neck Cancer Clinic.

Biobanks
**Trial biobank:** From each blood sample, 12 mL blood will be analyzed immediately, and the remaining blood will be stored as plasma at -80 degrees Celsius for batch analyses. These batch analyses will be performed before the project end date on January 31, 2020. This biobank will be established at the Department of Clinical Biochemistry, AUH.

**Biobank for future research:** Consent will be obtained from study participants for collection of extra blood at each blood sample time point, which can be used in future, unspecified research. Study participants who decline donating blood to this biobank for future research can still participate in the clinical trial. Collected material in the biobank for future research can only be used in future research projects after approval from the Ethics Committee and approval from the trial research team. The material will be stored at the Department of Clinical Biochemistry, AUH, for 15 years. After this time point leftover material will be destroyed.

Sample size calculation and statistics
An alpha (two-sided) of 0.05 and power (1 – beta) of 90 % have been applied for the sample size calculation. The primary endpoint is platelet aggregation measured by Multiplate®. From a previous study, the mean of the area under the curve for collagen-induced platelet aggregation was found to be 815 AU x min with standard deviation 130 AU x min [24]. We wish to detect a difference of at least 125 AU x min, which leads to inclusion of 23 patients in each of the two groups. We have chosen to include 30 patients in each group.

The difference in biochemical markers between groups over time will be tested using ANOVA models. The difference between groups at specific time points will be tested with the unpaired t-test for parametric data or the Wilcoxon rank-sum test for non-parametric data.

Ethical considerations
Study permission will be obtained from the Central Denmark Region’s Ethics Committee and the Danish Data Protection Agency. The Declaration of Helsinki will be followed in all aspects. Risks or adverse effects related to remote ischemic preconditioning have not previously been reported. Blood samples obtained during the day of surgery will be collected from an arterial cannula inserted by the anesthesiologist used for blood pressure monitoring during and after surgery. The blood sample obtained on the first postoperative day will be collected...
from that same arterial cannula, or from a central venous line or venipuncture if the arterial cannula has been removed. There is a small risk of developing hematoma or infection after venipuncture.

At each blood sample 28.8 mL blood are collected, or 36.4 mL blood if the study participant has given consent to the biobank for future research. Hence, 144 mL or 182 mL blood will be collected in total from each patient participating in the trial. The research team has assessed that collecting this total blood volume does not impose a risk on study participants.

All adverse effects related to the study intervention will be reported to the Ethics Committee.

The individual study participant will not directly benefit from participation in the trial. It is the research team’s opinion that new knowledge generated by this trial counterbalances the potential inconveniences and risks related to trial participation.

**Premature trial discontinuation**

The individual study participant will be excluded from the trial if he/she withdraws consent. The research team does not anticipate any circumstances where the whole trial might be discontinued.

**Data management**

Handling, analysis, and filing of data will be in compliance with the Danish Data Protection Act and regulations from the Danish Data Protection Agency. Identifiable data will be anonymized at the end of the trial. Data will be filed for 15 years after the end of the trial at the Department of Clinical Biochemistry, AUH.

**Disclosure of protected health information to the research team**

After informed consent has been obtained, clinical data will be collected during the trial from the patient’s electronic medical records. Only data described under “Study participants, methods and materials” will be collected.

**Funding**

The research team has initiated the trial. Aarhus University has granted a M.D./Ph.D. fellowship to Andreas Engel Krag. Running costs will be covered by grants from private foundations.

Study participants will not receive compensation for participating in the trial.

**Perspectives**

Total free flap necrosis occurs in up to 10% of cases, with potentially fatal consequences for patients who received head and neck reconstruction. The results of the trial will show if remote ischemic preconditioning reduces the risk of thrombosis, and thereby free flap loss in this group of patients.

**Feasibility**

Feasibility is secured in terms of patient recruitment and laboratory expertise. In 2013, 58 patients received microsurgical reconstruction at the Department of Plastic Surgery, AUH. The laboratory analyses will be performed at the accredited clinical laboratory and research unit at the Department of Clinical Biochemistry, AUH.
Publications
The results of the trial, whether positive, negative, or inconclusive, will be published in international peer-reviewed scientific journals.

Time line
The study period will be from September 1, 2015 to January 31, 2020. All patients are expected to be included in the clinical trial between 2015 and 2018, and laboratory analyses will be performed continually during the study period.

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