Prevention of myocardial injury by remote ischemic preconditioning in emergent or urgent non-cardiac surgery: a randomized clinical trial

The PIXIE study

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes (protocol).

2. Original statistical analysis plan, final statistical analysis plan, summary of changes (statistical analysis plan).

Prevention of myocardial injury by remote ischemic preconditioning in emergent or urgent non-cardiac surgery: a randomized clinical trial

The PIXIE study

Contact information

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Introduction

Worldwide, more than 200 million patients have major non-cardiac surgery annually and a significant proportion of these patients suffer major cardiovascular complications (e.g. nonfatal myocardial infarction, cardiac arrest, vascular death) within 30 days of their surgery [1, 2]. Perioperative myocardial infarction is the most common cardiovascular complication and recent clinical studies have shown that even minor myocardial injury in relation to non-cardiac surgery is associated with 30-day mortality [3, 4].

The pathogenesis of perioperative myocardial infarction is complex and not fully elucidated. Surgery is associated with trauma, anesthesia and analgesia, bleeding and anemia, pain, hypothermia and fasting [2]. These factors initiate systemic inflammation, hypercoagulability, increased levels of catecholamines and cortisol and a state of hypoxia [2]. The drastic systemic changes can lead to a myocardial hypoxic supply-demand mismatch where rupture of vulnerable coronary plaques might represent an additional mechanism [2, 5]. At the moment, no effective prevention or treatment of perioperative myocardial infarction in non-cardiac surgery is accepted.

The aim of this study is to determine whether an intervention with remote ischemic preconditioning can reduce myocardial injury in emergent or urgent non-cardiac surgery.

Remote ischemic preconditioning

Remote ischemic preconditioning is a procedure, which protects remote tissues and organs e.g. against ischemia-reperfusion injury [6]. Cycles of forearm or leg ischemia and reperfusion by the inflation of a blood-pressure cuff for brief periods are the preferred method [7]. The procedure is simple, safe and with no clear side effects [6].

The mechanism of remote ischemic preconditioning is not fully understood. However, it seems that the procedure triggers complex endogenous pathways including a humoral pathway, a neural pathway and a systemic anti-inflammatory pathway [7, 8].

In clinical trials covering acute cardiology and cardiac surgery, remote ischemic preconditioning has effectively reduced myocardial injury, postoperative cardiovascular complications and cardiac mortality [9, 10]. Recently, the effect of remote ischemic preconditioning on attenuating ischemia-reperfusion injury has been investigated in non-cardiac surgery [11-14]. The organ specific ischemia-reperfusion injury, systemic oxidative stress and inflammatory response were attenuated due to the intervention [11-14]. Whether

remote ischemic preconditioning protects against myocardial injury in the perioperative period of non-cardiac surgery have until now not been investigated.

Aim

- To determine whether remote ischemic preconditioning can reduce markers of myocardial injury in emergent or urgent non-cardiac surgery.

Methods

Trial design

Randomized clinical trial

Study setting

Department of Surgery, Køge Hospital, Denmark

Department of Orthopedic Surgery, Køge Hospital, Denmark

Department of Anesthesia, Køge Hospital, Denmark

Eligibility criteria

Inclusion criteria

- patients ≥ 45 years undergoing in-hospital major non-cardiac surgery
    - major general surgery
        - surgery with planned laparotomy or high risk of laparotomy or perforated intraperitoneal organ except the appendix.
    - major orthopedic surgery
        - hip surgery
- undergoing emergent or urgent surgery (i.e. emergent or urgent visitation to the Department of Surgery or Department of Orthopedic Surgery)
- fulfil 1 or more of the following 4 inclusion criteria, which are all determined during the conversation with the patient:

1. Ischemic heart disease, defined by any of the following criteria
    A. angina pectoris
    B. prior myocardial infarction
    C. prior percutaneous coronary intervention

D. prior coronary artery bypass graft

2. Peripheral arterial disease, defined by any of the following criteria

A. intermittent claudication

B. reduced peripheral arterial blood flow

C. prior vascular surgery due to peripheral arterial disease

3. Prior stroke

OR

4. any 1 of 7 risk criteria

A. age ≥70 years;

B. congestive heart failure

C. prior transient ischemic attack;

D. diabetes and currently taking an oral hypoglycemic agent or insulin

E. hypertension

G. preoperative serum creatinine >175 μmol/L (>2.0 mg/dl)

H. smoking within 2 years of surgery

Exclusion criteria

- History of peripheral arterial disease affecting both upper limbs
- Renal failure with eGRF<30ml/min/1.73m^2
- Cardiogenic shock or cardiac arrest during the current hospital admission
- Reoperation after elective surgery carried out during the current hospital admission
- Not capable of giving informed consent after oral and written information
- Previously included in this trial

Interventions

Remote ischemic preconditioning: 4 cycles of forearm ischemia (200 mmHg, 5 min) and reperfusion (5 min) immediately after induction of anesthesia. The procedure should be performed on the limb opposite the one used for iv infusions. For patients with systolic blood pressures >185 mmHg, the cuff will be inflated to at least 15 mmHg above the patient's systolic blood pressure.

Primary outcome

- Number of patients with peak plasma cardiac troponin I (cTnI) ≥ 45ng/L (99th percentile URL, 10% CV at 40ng/L) during surgery or during the first 4 days after surgery (perioperative myocardial injury). If elevated at baseline[1] (preoperative) a 20% or greater increase of cTnI according to baseline is required for the diagnosis of a perioperative myocardial injury [15].

Secondary outcome

- Peak plasma cTnI and total cTnI release (area under the curve) during surgery or during the first 4 days after surgery
- Endothelial dysfunction assessed by the non-invasive EndoPat system (24 hours and 72 hours after surgery)
- Perioperative myocardial infarction (Universal definition of myocardial infarction, published in 2012 [15])
    - A typical rise or fall of cTnI with peak plasma cTnI ≥ 45ng/L (99th percentile URL, 10% CV at 40 ng/L) and with at least one of the following:
        - Symptoms of ischemia
        - New or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block (LBBB)
        - Development of pathologic Q waves in the ECG
        - Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
        - Identification of an intracoronary thrombus by angiography or autopsy
    - Cardiac death with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes or new LBBB, but death occurred

[1] Defined as a preoperative cTnI ≥ 45ng/L (99th percentile URL, 10% CV at 40ng/L)

before cardiac biomarkers were obtained, or before cardiac biomarker values would be increased.

        - Development of new pathologic Q waves on an ECG or pathological findings of a healing myocardial infarction if troponin levels were obtained at times that could have missed the clinical event

- Major adverse cardiovascular events (30 days and one year after surgery)
    - Nonfatal cardiac arrest, coronary revascularization procedure (PCI or CABG), acute coronary syndrome, stroke, congestive heart failure, new clinically important cardiac arrhythmia, peripheral arterial thrombosis, rehospitalization for cardiovascular reasons.
- Length of postoperative hospital stay
- Length of intensive care unit stay
- All-cause mortality (30 days and one year after surgery)
    - cardiovascular or non-cardiovascular (a clearly documented non-cardiovascular cause)

Besides the inclusion and exclusion criteria, the following baseline data and preoperative/postoperative risk factors will be collected from each patient (records):

- Age, sex
- co-morbidities: chronic obstructive pulmonary disease, atrial fibrillation, active cancer
- medications (Paracetamol, NSAID, steroid, antibiotics, opioid, ASA, statin, brilique, clopidogrel, anticoagulant medications, β-blocker, calcium antagonist, diuretics, ACE-I, angiotensin antagonist, antiarytmic, nitrate, digoxin, antidiabetics incl. insulin) preoperative and during hospitalization
- preoperative ASA score
- preoperative revised cardiac risk index score
- type and length of surgery
- type and length of anesthesia and type of analgesia

- Intraoperative hypotension (and length) defined as a systolic blood pressure <100mmHg
- Preoperative and postoperative plasma creatinine (incl. eGFR) and plasma hemoglobin
- blood transfusion
- Clinical events during hospitalization according to the Clavien Dindo classification [16, 17] e.g. pneumonia, sepsis, acute respiratory failure, acute kidney failure and lung embolism.

Participant timeline

Intervention: The remote ischemic preconditioning procedure is carried out immediately after the induction of regional or general anesthesia. The blood pressure cuff is placed on the upper limb. The cuff is inflated to 200 mmHg resulting in a total occlusion of the blood flow to the limb. After 5 minutes of ischemia the cuff is deflated and the limb is reperfused for 5 minutes. The procedure is carried out 4 times in a row. For patients with systolic blood pressures >185 mmHg, the cuff will be inflated to at least 15 mmHg above the patient's systolic blood pressure.

The patients will have blood collected to assess cTnI (preoperative and in the morning at days 1, 2, 3 and 4 after surgery). All patients with a cTnI assessment ≥ 45ng/L will have a minimum of one ECG performed and a medical doctor will evaluate the patient for the presence of an ischemic or non-ischemic event that can explain the elevated cTnI (i.e. sepsis, pulmonary embolism, cardioversion). The medical doctor will determine whether further action should be taken. If necessary a cardiologist will be consulted.

The investigator observes the patients during their stay in hospital and records the occurrence of any primary or secondary outcomes. The investigator will undertake a short- and long-term follow up (30 days and 1 year after surgery) and record the occurrence of any primary or secondary outcomes. The follow-up will be carried out by reviewing the patients' electronic hospital records and by a personal phone call.

Sample size: A large international cohort study done by the VISION investigator group showed that 8.0% of patients undergoing non-cardiac surgery suffered myocardial injury [4]. In our

study we include patients with a moderate – high risk of suffering myocardial injury, while the VISION study included patients at all risk levels. We predict that 15% of the patients in the placebo group will suffer from myocardial injury and that the incidence of myocardial injury will be reduced to 7% in the intervention group. Type I error is set at 5% and type II error is set at 20%. In total 2 x 264 patients need to be included based on this power calculation. We will include patients until we have a total of 2 x 270 patients for evaluation.

We will do an interim analysis based on the primary outcome when a total of 270 (50%) evaluable patients have been included in the study. We will use the O'Brien Fleming method with a level of significance at $p<0.05$ (first analysis) and $p<0.048$ (second analysis).

Recruitment

All patients hospitalized to undergo emergent or urgent major noncardiac surgery at Department of Surgery and Department of Orthopedic Surgery, Køge Hospital, will as far as possible be screened to determine whether they meet the inclusion and exclusion criteria.

Allocation and blinding

An electronic generator will create the randomization list.

Each patient allocation is randomly selected using sealed opaque envelopes. It is not possible to blind the staff or the patient. Assessor blinding will be performed and the unblinding will take place after final data analysis has been made. An independent data analysis team consisting of a statistician and an external medical scientist will perform interim analysis.

Data collection methods

<u>Blood samples</u>

The blood will be collected from a larger vein (e.g. the cubital vein).

<u>Plasma cardiac troponin I</u>

Plasma cTnI will be analyzed at the local department of biochemistry using Siemens Healthcare Dimension Vista.

ECG

Trained healthcare professionals using standard ECG equipment will record the ECG.

Withdrawal criteria and dropouts

The patient can at any time decide to discontinue his/hers participation in the trial. In case of major serious per- or postoperative complications/events, the investigator can decide to withdraw the patient from the trial for the sake of the patient.

The number of dropouts and withdrawals will be reported in the final manuscript and new participants will be included until 2 x 270 patients have completed the trial. In order to complete the protocol the patient must have received the intervention/placebo and have a minimum of two postoperative cTnI assessments done within the first 4 postoperative days (the primary outcome). If the primary outcome is missing the short- and long-term follow-up will still be done and the patient will be included in the final analysis (intention-to-treat analysis). The patient will dropout if reoperated within the first 4 postoperative days.

Data

Data will be collected according to "Datatilsynet" and "Persondataloven". The trial will be approved by the regional ethics committee, and the trial will be registered on www.clinicaltrials.gov before inclusion of the first patient. All collected data will be registered in case report forms (CRFs) according to a list of source data. We will use paper CRFs, and later all data will be entered into an electronic database for statistical analysis. Electronic data will according to the approval by "Datatilsynet" be stored at an access limited drive.

Statistics

All statistical analysis will be done with SPSS/PC+ package (SSPS, Chicago, IL, USA) or the SAS 9.3 package. Data will be analyzed using non-parametric or parametric statistics depending on the distribution of data. Differences are considered significant when $p < 0.048$ (final analysis). We will do a per-protocol analysis and an intention-to-treat analysis.

A medical doctor will evaluate all patients with a rise in cTnI ≥ 45ng/L in order to determine whether the elevation in cTnI is due to an ischemic or non-ischemic disease. In the

final data analysis we will do an analysis, which includes all patients (ischemic and non-ischemic) and a second analysis, which will only include patients with an ischemic disease.

Ethics and dissemination

Informed consent

Potential participants will be contacted personally prior to the operation by the anaesthesiologist, who is in charge of the operation. The patients will receive oral and written information regarding:

- The trial, and that we would like to ask he/she to participate in the trial.
- That it is voluntary to participate, and the subject has the right to and is encouraged to take time to consider carefully if he/she wants to participate.
- The subject has the right to bring a friend or family member to the oral participant information
- That the trial is approved by "Datatilsynet" and "den Videnskabsetiske Komité"
- That the subject at any time can cease to participate in the trial and if they do it will not have any consequences for further treatment

The meeting will take place in a calm and quiet environment. Since all included patients require emergent or urgent surgery, it will not in all cases be possible to give the patient 24 hours to consider participation. However, all patients will be given enough time to read the written information, listen to the oral information and ask any questions. A similar setup is required in a randomized clinical trial, which we are currently conducting. The study includes patients in the acute phase of an acute myocardial infarction and was approved by "Den Videnskabsetiske Komité, Region Hovedstaden" (Journal nummer: H-3-2010-117, EudraCT nummer: 2010-022400-53).
In the written information the amendment "Dine rettigheder som forsøgsperson i et biomedicinsk forskningsprojekt" from "Den Centrale Videnskabsetiske Komités" pre-printed

folder will be included. The patient will be encouraged to read this before giving his informed consent. It is central and important to investigate the remote ischemic preconditioning intervention in a group of acute patients. Patients that require acute surgery have a much higher risk than others for developing major cardiovascular complication. Therefore, it is of particular importance to investigate if this non-invasive intervention can improve the treatment and outcome for this group of high-risk patients.

Safety

The use of a blood pressure cuff/tourniquet in remote ischemic preconditioning is considered completely safe. There is a small possibility of having skin erythema in the area where the inflated cuff was placed. In case of regional anesthesia the patient can experience numbness and discomfort during the preconditioning procedure. The discomfort will be similar to a 5-minute blood pressure assessment. An identical preconditioning protocol was used in prior Danish clinical trials including patients with stroke [18] and acute myocardial infarction [19]. In one of the studies a questionnaire was designed to detect any discomfort created by the preconditioning [18]. No reports of significant discomfort were reported [18]. The numbness and skin erythema are both fully reversible and will rapidly wear off. There is a very small possibility of getting a skin infection or haematoma after having the blood samples taken. We will therefore use a standardised aseptic technique in trying to avoid this complication.

Ethical considerations

We seek the approval from “den Videnskabsetiske Komité” and “Datatilsynet”.

The ischemic intervention is considered completely safe, and has been done in previous large clinical trials [9, 10]. No serious adverse events have been reported [9, 10]. The patients will gain a personal benefit by participating if this simple and low-risk intervention is effective. The risk of myocardial injury and postoperative cardiovascular morbidity could be markedly reduced in the intervention group. The preconditioning procedure will not prolong the anesthetic time or delay the onset of surgery.

Participation in the trial is voluntary. Patients will be given oral and written information according to the Helsinki II declaration. The patients can, at any time, withdraw from the

project, which will have no consequences for further treatment. The authorized healthcare professionals involved in the project will demonstrate diligence in their duties. The participating patients' integrity and autonomy will be respected. The trial will be carried out at the Department of Surgery, Køge Hospital.

Duration:

The trial is planned to initiate when all relevant approvals have been received ("Datatilsynet", "den Videnskabsetiske Komité"). We expect to include 4-6 subjects each week. We expect to start the trial in January 2014 and end it in January 2016.

Economy:

The patients will not receive any economic compensation for their participation.

The trial is initiated by: Sarah Ekeløf Busch (MD, PhD student), Ismail Gögenur (MD, professor, DMSc) from Department of Surgery, Køge Hospital and Ole Mathiesen (MD, Ph.D.) from Department of Anesthesiology, Køge Hospital.

Funding

The trial has received funding from:

Toyota Fonden	100.000kr
Beckett-Fonden	100.000kr
Snedkermester Sophus Jacobsens Fond	30.000kr

The study was designed and will be conducted, analysed, interpreted, and reported independently of the funding sources.

Publication

All results, positive as well as negative will be published in international journals with peer-review. Authors will all fulfill the ICMJE-guidelines for authorship.

Order of authors in all publications

1st author: Sarah Ekeløf Busch

2nd author: Ole Mathiesen

3rd author: Mikkel Holm Larsen

Last author: Ismail Gögenur

More authors may be added if they according to the authors listed fulfill the ICMJE-guidelines for authorship.

The possible significance of the trial

Perioperative myocardial infarction and myocardial injury in noncardiac surgery is more common than previously anticipated. At the moment no prevention or treatment is known and universally accepted. Remote ischemic preconditioning is a safe, cheap and a fast intervention, which can be easily implemented. If the intervention is effective we can potentially reduce the perioperative and postoperative cardiovascular morbidity and mortality.

Referencer

1. Weiser, T.G., et al., *An estimation of the global volume of surgery: a modelling strategy based on available data.* Lancet 2008;**372**:139-44.
2. Devereaux, P.J., et al., *Perioperative cardiac events in patients undergoing noncardiac surgery: a review of the magnitude of the problem, the pathophysiology of the events and methods to estimate and communicate risk.* Cmaj 2005;**176:**627-34.
3. Devereaux, P.J., et al., *Effects of extended-release metoprolol succinate in patients undergoing non-cardiac surgery (POISE trial): a randomised controlled trial.* Lancet 2008;**371:**1839-47.
4. Devereaux, P.J., et al., *Association between postoperative troponin levels and 30-day mortality among patients undergoing noncardiac surgery.* Jama 2012;**307**:2295-304.
5. Landesberg, G., et al., *Perioperative myocardial infarction.* Circulation 2009;**119**:2936-44.
6. Kharbanda, R.K., T.T. Nielsen, and A.N. Redington, *Translation of remote ischaemic preconditioning into clinical practice.* Lancet 2009;**374**:1557-65.
7. Hausenloy, D.J. and D.M. Yellon, *Remote ischaemic preconditioning: underlying mechanisms and clinical application.* Cardiovasc Res 2008;**79**:377-86.
8. Tapuria, N., et al., *Remote ischemic preconditioning: a novel protective method from ischemia reperfusion injury--a review.* J Surg Res 2008;**150**:304-30.
9. Thielmann, M., et al., *Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial.* Lancet 2013;**382**:597-604.
10. Botker, H.E., et al., *Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial.* Lancet, 2010;**375**:727-34.
11. Li, C., et al., *Limb remote ischemic preconditioning for intestinal and pulmonary protection during elective open infrarenal abdominal aortic aneurysm repair: a randomized controlled trial.* Anesthesiology 2013;**118**:842-52.
12. Li, C., et al., *Limb Remote Ischemic Preconditioning Attenuates Lung Injury after Pulmonary Resection under Propofol-Remifentanil Anesthesia: A Randomized Controlled Study.* Anesthesiology 2014.
13. Hu, S., et al., *Effects of remote ischemic preconditioning on biochemical markers and neurologic outcomes in patients undergoing elective cervical decompression surgery: a prospective randomized controlled trial.* J Neurosurg Anesthesiol 2010;**22**:46-52.
14. Huang, J., et al., *Effect of remote ischaemic preconditioning on renal protection in patients undergoing laparoscopic partial nephrectomy: a 'blinded' randomised controlled trial.* BJU Int 2013;**112**:74-80.
15. Thygesen, K., et al., *Third universal definition of myocardial infarction.* J Am Coll Cardiol 2012;**60**:1581-98.
16. *A clinical trial comparing primary coronary angioplasty with tissue plasminogen activator for acute myocardial infarction. The Global Use of Strategies to Open Occluded Coronary Arteries in Acute Coronary Syndromes (GUSTO IIb) Angioplasty Substudy Investigators.* N Engl J Med 1997;**336**:1621-8.
17. Dindo, D., N. Demartines, and P.A. Clavien, *Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey.* Ann Surg 2004;**240**:205-13.
18. Hougaard, K.D., et al., *Remote ischemic perconditioning as an adjunct therapy to thrombolysis in patients with acute ischemic stroke: a randomized trial.* Stroke 2014;**45**:159-67.
19. Sloth, A.D., et al., *Improved long-term clinical outcomes in patients with ST-elevation myocardial infarction undergoing remote ischaemic conditioning as an adjunct to primary percutaneous coronary intervention.* Eur Heart J 2014;**35**:168-75.

Prevention of myocardial injury by remote ischemic preconditioning in emergent or urgent non-cardiac surgery: a randomized clinical trial

The PIXIE study

Contact information

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Introduction

Worldwide, more than 200 million patients have major non-cardiac surgery annually and a significant proportion of these patients suffer major cardiovascular complications (e.g. nonfatal myocardial infarction, cardiac arrest, vascular death) within 30 days of their surgery [1, 2]. Perioperative myocardial infarction is the most common cardiovascular complication and recent clinical studies have shown that even minor myocardial injury in relation to non-cardiac surgery is associated with 30-day mortality [3, 4].

The pathogenesis of perioperative myocardial infarction is complex and not fully elucidated. Surgery is associated with trauma, anesthesia and analgesia, bleeding and anemia, pain, hypothermia and fasting [2]. These factors initiate systemic inflammation, hypercoagulability, increased levels of catecholamines and cortisol and a state of hypoxia [2]. The drastic systemic changes can lead to a myocardial hypoxic supply-demand mismatch where rupture of vulnerable coronary plaques might represent an additional mechanism [2, 5]. At the moment, no effective prevention or treatment of perioperative myocardial infarction in non-cardiac surgery is accepted.

The aim of this study is to determine whether an intervention with remote ischemic preconditioning can reduce myocardial injury in emergent or urgent non-cardiac surgery.

Remote ischemic preconditioning

Remote ischemic preconditioning is a procedure, which protects remote tissues and organs e.g. against ischemia-reperfusion injury [6]. Cycles of forearm or leg ischemia and reperfusion by the inflation of a blood-pressure cuff for brief periods are the preferred method [7]. The procedure is simple, safe and with no clear side effects [6].

The mechanism of remote ischemic preconditioning is not fully understood. However, it seems that the procedure triggers complex endogenous pathways including a humoral pathway, a neural pathway and a systemic anti-inflammatory pathway [7, 8].

In clinical trials covering acute cardiology and cardiac surgery, remote ischemic preconditioning has effectively reduced myocardial injury, postoperative cardiovascular complications and cardiac mortality [9, 10]. Recently, the effect of remote ischemic preconditioning on attenuating ischemia-reperfusion injury has been investigated in non-cardiac surgery [11-14]. The organ specific ischemia-reperfusion injury, systemic oxidative

stress and inflammatory response were attenuated due to the intervention [11-14]. Whether remote ischemic preconditioning protects against myocardial injury in the perioperative period of non-cardiac surgery have until now not been investigated.

Aim

- To determine whether remote ischemic preconditioning can reduce markers of myocardial injury in emergent or urgent non-cardiac surgery.

Methods

Trial design

Randomized clinical trial

Study setting

Department of Surgery, Zealand University Hospital, Denmark
Department of Orthopedic Surgery, Zealand University Hospital, Denmark
Department of Anesthesiology, Zealand University Hospital, Denmark
Department of Orthopedic Surgery, Holstebro Hospital, Denmark
Department of Clinical Biochemistry, Holstebro Hospital, Denmark
Department of Anesthesiology, Herlev Hospital, Denmark
Department of Orthopedic Surgery, Herlev Hospital, Denmark

Eligibility criteria

Inclusion criteria

- patients ≥ 45 years undergoing in-hospital major non-cardiac surgery
    - major orthopedic surgery
        - hip surgery
- undergoing emergent or urgent surgery (i.e. emergent or urgent visitation to the Department of Orthopedic Surgery)
- fulfil 1 or more of the following 4 inclusion criteria, which are all determined during the conversation with the patient:
    1. Ischemic heart disease, defined by any of the following criteria
        A. angina pectoris

B. prior myocardial infarction

C. prior percutaneous coronary intervention

D. prior coronary artery bypass graft

2. Peripheral arterial disease, defined by any of the following criteria

A. intermittent claudication

B. reduced peripheral arterial blood flow

C. prior vascular surgery due to peripheral arterial disease

3. Prior stroke

OR

4. any 1 of 7 risk criteria

A. age ≥70 years;

B. congestive heart failure

C. prior transient ischemic attack;

D. diabetes and currently taking an oral hypoglycemic agent or insulin

E. hypertension

G. preoperative serum creatinine >175 μmol/L (>2.0 mg/dl)

H. smoking within 2 years of surgery

Exclusion criteria

- History of peripheral arterial disease affecting both upper limbs
- Renal failure with eGRF<30ml/min/1.73m^2
- Cardiogenic shock or cardiac arrest during the current hospital admission
- Reoperation after elective surgery carried out during the current hospital admission
- Other conditions that prevent the performance of remote ischemic preconditioning.
- Not capable of giving informed consent after oral and written information
- Previously included in this trial

Interventions

Remote ischemic preconditioning: 4 cycles of forearm ischemia (200 mmHg, 5 min) and reperfusion (5 min) immediately after induction of anesthesia. The procedure should be performed on the limb opposite the one used for iv infusions. For patients with systolic blood

pressures >185 mmHg, the cuff will be inflated to at least 15 mmHg above the patient's systolic blood pressure.

Primary outcome

- Number of patients with peak plasma cardiac troponin I (cTnI) ≥ 45ng/L (99th percentile URL, 10% CV at 40ng/L) during surgery or during the first 4 days after surgery (perioperative myocardial injury). If elevated at baseline[1] (preoperative) a 20% or greater increase of cTnI according to baseline is required for the diagnosis of a perioperative myocardial injury [15].
    - Holstebro Hospital: Number of patients with peak plasma cardiac troponin I (cTnI) > 24 ng/L (99th percentile URL, 14% CV)

Secondary outcome

- Peak plasma cTnI and total cTnI release (area under the curve) during surgery or during the first 4 days after surgery
- Plasma N-terminal Pro-Brain Natriuretic Peptide (NT-proBNP) before surgery and at day 1 after surgery.
- Endothelial dysfunction assessed by the non-invasive EndoPat system (24 hours after surgery, only done at Zealand University Hospital)
- Biomarkers of coagulation (only patients included at Holstebro Hospital)
- Perioperative myocardial infarction (Universal definition of myocardial infarction, published in 2012 [15])
    - A typical rise or fall of cTnI with peak plasma cTnI ≥ 45ng/L (99th percentile URL, 10% CV at 40 ng/L) (Holstebro cTnI > 24 ng/L), and with at least one of the following:
        - Symptoms of ischemia

[1] Defined as a preoperative cTnI ≥ 45ng/L (99th percentile URL, 10% CV at 40ng/L)

- New or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block (LBBB)
- Development of pathologic Q waves in the ECG
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
- Identification of an intracoronary thrombus by angiography or autopsy

- Cardiac death with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes or new LBBB, but death occurred before cardiac biomarkers were obtained, or before cardiac biomarker values would be increased.
- Development of new pathologic Q waves on an ECG or pathological findings of a healing myocardial infarction if troponin levels were obtained at times that could have missed the clinical event

- Major adverse cardiovascular events (30 days, one year and five years after surgery)
    - Nonfatal cardiac arrest, coronary revascularization procedure (PCI or CABG), acute coronary syndrome, stroke, congestive heart failure, new clinically important cardiac arrhythmia, peripheral arterial thrombosis, rehospitalization for cardiovascular reasons.
- Length of postoperative hospital stay
- Length of intensive care unit stay
- All-cause mortality (30 days, one year and five years after surgery)
    - cardiovascular or non-cardiovascular (a clearly documented non-cardiovascular cause)

Besides the inclusion and exclusion criteria, the following baseline data and preoperative/postoperative risk factors will be collected from each patient (records):

- Age, sex

- co-morbidities: chronic obstructive pulmonary disease, atrial fibrillation, active cancer, autoimmune diseases
- medications (Paracetamol, NSAID, steroid, antibiotics, opioid, ASA, statin, brilique, clopidogrel, anticoagulant medications, β-blocker, calcium antagonist, diuretics, ACE-I, angiotensin antagonist, antiarytmic, nitrate, digoxin, antidiabetics incl. insulin) preoperative and during hospitalization
- preoperative ASA score
- preoperative revised cardiac risk index score
- type and length of surgery
- type and length of anesthesia and type of analgesia
- Intraoperative hypotension (and length) defined as a systolic blood pressure <100mmHg
- Preoperative and postoperative plasma creatinine (incl. eGFR) and plasma hemoglobin
- blood transfusion
- Clinical events during hospitalization according to the Clavien Dindo classification [16, 17] e.g. pneumonia, sepsis, acute respiratory failure, acute kidney failure and lung embolism.

The one and five year follow-up on all included patients will be performed with information (procedure- and diagnosis codes) registered in the Danish National Patients Register (Landspatientsregisteret). Likewise, information on death and cause of death will be deducted from "Dødsårsagsregisteret".

Participant timeline

Intervention: The remote ischemic preconditioning procedure is carried out immediately after the induction of regional or general anesthesia. The blood pressure cuff is placed on the upper limb. The cuff is inflated to 200 mmHg resulting in a total occlusion of the blood flow to the limb. After 5 minutes of ischemia the cuff is deflated and the limb is reperfused for 5 minutes. The procedure is carried out 4 times in a row. For patients with systolic blood pressures >185 mmHg, the cuff will be inflated to at least 15 mmHg above the patient's systolic blood pressure. The first round should be completed before the initiation of surgery.

The patients will have blood collected to assess cTnI (preoperative and in the morning at days 1, 2, 3 and 4 after surgery) and NT proBNP (preoperative and in the morning at day 1 after surgery). All patients with a cTnI assessment ≥ 45ng/L will have a minimum of one ECG performed and a medical doctor will evaluate the patient for the presence of an ischemic or non-ischemic event that can explain the elevated cTnI (i.e. sepsis, pulmonary embolism, cardioversion). The medical doctor will determine whether further action should be taken. If necessary, a cardiologist will be consulted. Patient included at Holstebro Hospital will have blood collected for assessment of coagulation biomarkers. The blood samples are taken preoperatively, 2 hours after incision and in the morning at day 1 and 2 after surgery (4 times 6 ml blood).

The investigator observes the patients during their stay in hospital and records the occurrence of any primary or secondary outcomes. The investigator will undertake a short- and long-term follow up (30 days, one and five years after surgery) and record the occurrence of any primary or secondary outcomes. The follow-up will be carried out by reviewing the patients' electronic hospital records, by a personal phone call and with data from the Danish National Patient Register and "Dødsårsagsregisteret".

Sample size: A large international cohort study done by the VISION investigator group showed that 8.0% of patients undergoing non-cardiac surgery suffered myocardial injury [4]. In our study we include patients with a moderate – high risk of suffering myocardial injury, while the VISION study included patients at all risk levels. We predict that 15% of the patients in the placebo group will suffer from myocardial injury and that the incidence of myocardial injury will be reduced to 7% in the intervention group. Type I error is set at 5% and type II error is set at 20%. In total 2 x 264 patients need to be included based on this power calculation. We will include patients until we have a total of 2 x 270 patients for evaluation.

Biobank

We will establish a biobank at Department of Clinical Biochemistry, Holstebro Hospital, containing the drawn plasma for analysis of biomarkers of coagulation and NT-pro-BNP. Only patients included at Holstebro Hospital will have samples drawn for the biobank. In total, a

maximum of 30 ml blood will be drawn from each participating patient (24mL for the coagulation biomarkers and 6 ml for NT-pro-BNP). The blood samples will be centrifuged and only plasma samples will be stored (-80^{0}C). Moreover, a biobank will be established at the Department of Surgery, Zealand University Hospital. Every 4-6 month plasma samples for NT-pro-BNP analysis will transferred securely from the biobank at Holstebro Hospital to the biobank at Zealand University Hospital. All plasma samples will be retained until all analyzes are made and for no more than 10 years. The purpose of the biobank is to make sure that all assessments are made with the same equipment in the lab, hereby reducing variations due to technical variations in the equipment used. All requirements regarding establishment and maintenance of the biobank will be met.

Recruitment

All patients hospitalized to undergo emergent or urgent major noncardiac surgery at Department of Orthopedic Surgery, Zealand University Hospital, Department of Orthopedic Surgery, Holstebro Hospital, and Department of Orthopedic Surgery, Herlev Hospital, will as far as possible be screened to determine whether they meet the inclusion and exclusion criteria.

Allocation and blinding

An electronic generator will create the randomization list.
Each patient allocation is randomly selected using sealed opaque envelopes. It is not possible to blind the staff or the patient. Assessor blinding will be performed and the unblinding will take place after final data analysis has been made. An independent data analysis team consisting of a statistician and an external medical scientist will perform interim analysis.

Data collection methods

<u>Blood samples</u>

The blood will be collected from a larger vein (e.g. the cubital vein).

Plasma cardiac troponin I

Plasma cTnI will be analyzed at the local department of biochemistry using Siemens Healthcare Dimension Vista (Zealand University Hospital, Herlev Hospital), Abbott high-sensitive troponin I (Holstebro Hospital).

Plasma NT proBNP

Plasma NT proBNP will be analyzed in a standardized manner at the Department of Biochemistry. Samples from Holstebro Hospital will be analyzed as one batch in Region Zealand when all patients have been included. Until then the plasma samples will be stored in a biobank at Department of Biochemistry (Holstebro Hospital) and Department of Surgery (Zealand University Hospital).

Coagulation biomarkers

The blood is centrifuged and the remaining plasma is stored in the bio-bank at -80^{O}C until further analysis.

ECG

Trained healthcare professionals using standard ECG equipment will record the ECG.

Withdrawal criteria and dropouts

The patient can at any time decide to discontinue his/hers participation in the trial. In case of major serious per- or postoperative complications/events, the investigator can decide to withdraw the patient from the trial for the sake of the patient.

The number of dropouts and withdrawals will be reported in the final manuscript and new participants will be included until 2 x 270 patients have completed the trial. In order to complete the protocol the patient must have received the intervention/placebo and have a minimum of two postoperative cTnI assessments done within the first 4 postoperative days (the primary outcome). If the primary outcome is missing the short- and long-term follow-up will still be done and the patient will be included in the final analysis (intention-to-treat analysis). The patient will dropout if reoperated within the first 4 postoperative days.

Data

Data will be collected according to "Datatilsynet" and "Persondataloven". The trial will be approved by the regional ethics committee, and the trial will be registered on www.clinicaltrials.gov before inclusion of the first patient. All collected data will be registered in case report forms (CRFs) according to a list of source data. We will use paper CRFs, and later all data will be entered into an electronic database for statistical analysis. Electronic data will according to the approval by "Datatilsynet" be stored at an access limited drive.

Statistics

All statistical analysis will be done with SPSS/PC+ package (SSPS, Chicago, IL, USA) or the SAS 9.3 package. Data will be analyzed using non-parametric or parametric statistics depending on the distribution of data. Differences are considered significant when $p < 0.05$. We will do a per-protocol analysis and an intention-to-treat analysis.

A medical doctor will evaluate all patients with a rise in cTnI ≥ 45ng/L in order to determine whether the elevation in cTnI is due to an ischemic or non-ischemic disease. In the final data analysis, we will do an analysis, which includes all patients (ischemic and non-ischemic) and a second analysis, which will only include patients with an ischemic disease.

Ethics and dissemination

Informed consent

Potential participants will be contacted personally prior to the operation by the anaesthesiologist or surgeon, who is in charge of the operation. The patients will receive oral and written information regarding:

- The trial, and that we would like to ask he/she to participate in the trial.
- That it is voluntary to participate, and the subject has the right to and is encouraged to take time to consider carefully if he/she wants to participate.

- The subject has the right to bring a friend or family member to the oral participant information
- That the trial is approved by "Datatilsynet" and "den Videnskabsetiske Komité"
- That the subject at any time can cease to participate in the trial and if they do it will not have any consequences for further treatment
- Patient at Holstebro Hospital: We have established a biobank at Department of Clinical Biochemistry (Holstebro Hospital) and Department of Surgery (Zealand University Hospital). Their plasma for assessment of coagulation markers and NT-pro-BNP will be stored until further analysis.

The meeting will take place in a calm and quiet environment. Since all included patients require emergent or urgent surgery, it will not in all cases be possible to give the patient 24 hours to consider participation. However, all patients will be given enough time to read the written information, listen to the oral information and ask any questions. A similar setup is required in a randomized clinical trial, which we are currently conducting. The study includes patients in the acute phase of an acute myocardial infarction and was approved by "Den Videnskabsetiske Komité, Region Hovedstaden" (Journal nummer: H-3-2010-117, EudraCT nummer: 2010-022400-53).

In the written information the amendment "Dine rettigheder som forsøgsperson i et biomedicinsk forskningsprojekt" from "Den Centrale Videnskabsetiske Komités" pre-printed folder will be included. The patient will be encouraged to read this before giving his informed consent. It is central and important to investigate the remote ischemic preconditioning intervention in a group of acute patients. Patients that require acute surgery have a much higher risk than others for developing major cardiovascular complication. Therefore, it is of particular importance to investigate if this non-invasive intervention can improve the treatment and outcome for this group of high-risk patients.

Safety

The use of a blood pressure cuff/tourniquet in remote ischemic preconditioning is considered completely safe. There is a small possibility of having skin erythema in the area where the inflated cuff was placed. In case of regional anesthesia, the patient can experience numbness and discomfort during the preconditioning procedure. The discomfort will be similar to a 5-minute blood pressure assessment. An identical preconditioning protocol was used in prior Danish clinical trials including patients with stroke [18] and acute myocardial infarction [19]. In one of the studies a questionnaire was designed to detect any discomfort created by the preconditioning [18]. No reports of significant discomfort were reported [18]. The numbness and skin erythema are both fully reversible and will rapidly wear off. There is a very small possibility of getting a skin infection or haematoma after having the blood samples taken. We will therefore use a standardised aseptic technique in trying to avoid this complication.

Ethical considerations

We seek the approval from "den Videnskabsetiske Komité" and "Datatilsynet".

The ischemic intervention is considered completely safe, and has been done in previous large clinical trials [9, 10]. No serious adverse events have been reported [9, 10]. The patients will gain a personal benefit by participating if this simple and low-risk intervention is effective. The risk of myocardial injury and postoperative cardiovascular morbidity could be markedly reduced in the intervention group. The preconditioning procedure will not prolong the anesthetic time or delay the onset of surgery.

Participation in the trial is voluntary. Patients will be given oral and written information according to the Helsinki II declaration. The patients can, at any time, withdraw from the project, which will have no consequences for further treatment. The authorized healthcare professionals involved in the project will demonstrate diligence in their duties. The participating patients' integrity and autonomy will be respected. The trial will be carried out at the Department of Surgery, Zealand University Hospital.

Duration:

The trial is planned to initiate when all relevant approvals have been received ("Datatilsynet", "den Videnskabsetiske Komité"). We expect to include 4-6 subjects each week. We expect to start the trial in January 2014 and end it in January 2018.

Economy:

The patients will not receive any economic compensation for their participation.
The trial is initiated by: Sarah Ekeløf Busch (MD, PhD student), Ismail Gögenur (MD, professor, DMSc) from Department of Surgery, Zealand University Hospital and Ole Mathiesen (MD, Ph.D.) from Department of Anesthesiology, Zealand University Hospital.

Funding

The trial has received funding from:

Toyota Fonden	100.000kr
Beckett-Fonden	100.000kr
Snedkermester Sophus Jacobsens Fond	30.000kr

The study was designed and will be conducted, analyzed, interpreted, and reported independently of the funding sources.

Budget

EXPENSES	**SPECIFICATION**	**UNIT**	**PER UNIT/KR**	**TOTAL/KR**
EQUIPMENT	Endopat 2000 (Itamar Medical Ltd., Caesarea, Israel)	1	161,250	161,250
	Finger probes	2160	1,980kr/box with 12 units (180 boxes)	356,400
ANALYSIS	Troponin I	2700	30	81,000
	Pro-BNP	1080	180	194,400
WAGES	Scholarship			120,000
SUM				913,050
OVERHEAD (3.1%)				28,304.55
TOTAL				**941,354.55**

Publication

All results, positive as well as negative will be published in international journals with peer-review. Authors will all fulfill the ICMJE-guidelines for authorship.

Order of authors in all publications

1st author: Sarah Ekeløf Busch

2nd author: Ole Mathiesen

Last author: Ismail Gögenur

More authors may be added if they according to the authors listed fulfill the ICMJE-guidelines for authorship.

The possible significance of the trial

Perioperative myocardial infarction and myocardial injury in noncardiac surgery is more common than previously anticipated. At the moment no prevention or treatment is known and universally accepted. Remote ischemic preconditioning is a safe, cheap and a fast intervention, which can be easily implemented. If the intervention is effective, we can potentially reduce the perioperative and postoperative cardiovascular morbidity and mortality.

Referencer

1. Weiser, T.G., et al., *An estimation of the global volume of surgery: a modelling strategy based on available data.* Lancet 2008;**372**:139-44.
2. Devereaux, P.J., et al., *Perioperative cardiac events in patients undergoing noncardiac surgery: a review of the magnitude of the problem, the pathophysiology of the events and methods to estimate and communicate risk.* Cmaj 2005;**176:**627-34.
3. Devereaux, P.J., et al., *Effects of extended-release metoprolol succinate in patients undergoing non-cardiac surgery (POISE trial): a randomised controlled trial.* Lancet 2008;**371:**1839-47.
4. Devereaux, P.J., et al., *Association between postoperative troponin levels and 30-day mortality among patients undergoing noncardiac surgery.* Jama 2012;**307**:2295-304.
5. Landesberg, G., et al., *Perioperative myocardial infarction.* Circulation 2009;**119**:2936-44.
6. Kharbanda, R.K., T.T. Nielsen, and A.N. Redington, *Translation of remote ischaemic preconditioning into clinical practice.* Lancet 2009;**374**:1557-65.
7. Hausenloy, D.J. and D.M. Yellon, *Remote ischaemic preconditioning: underlying mechanisms and clinical application.* Cardiovasc Res 2008;**79**:377-86.
8. Tapuria, N., et al., *Remote ischemic preconditioning: a novel protective method from ischemia reperfusion injury--a review.* J Surg Res 2008;**150**:304-30.
9. Thielmann, M., et al., *Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial.* Lancet 2013;**382**:597-604.
10. Botker, H.E., et al., *Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial.* Lancet, 2010;**375**:727-34.
11. Li, C., et al., *Limb remote ischemic preconditioning for intestinal and pulmonary protection during elective open infrarenal abdominal aortic aneurysm repair: a randomized controlled trial.* Anesthesiology 2013;**118**:842-52.
12. Li, C., et al., *Limb Remote Ischemic Preconditioning Attenuates Lung Injury after Pulmonary Resection under Propofol-Remifentanil Anesthesia: A Randomized Controlled Study.* Anesthesiology 2014.
13. Hu, S., et al., *Effects of remote ischemic preconditioning on biochemical markers and neurologic outcomes in patients undergoing elective cervical decompression surgery: a prospective randomized controlled trial.* J Neurosurg Anesthesiol 2010;**22**:46-52.
14. Huang, J., et al., *Effect of remote ischaemic preconditioning on renal protection in patients undergoing laparoscopic partial nephrectomy: a 'blinded' randomised controlled trial.* BJU Int 2013;**112**:74-80.
15. Thygesen, K., et al., *Third universal definition of myocardial infarction.* J Am Coll Cardiol 2012;**60**:1581-98.
16. *A clinical trial comparing primary coronary angioplasty with tissue plasminogen activator for acute myocardial infarction. The Global Use of Strategies to Open Occluded Coronary Arteries in Acute Coronary Syndromes (GUSTO IIb) Angioplasty Substudy Investigators.* N Engl J Med 1997;**336**:1621-8.
17. Dindo, D., N. Demartines, and P.A. Clavien, *Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey.* Ann Surg 2004;**240**:205-13.
18. Hougaard, K.D., et al., *Remote ischemic perconditioning as an adjunct therapy to thrombolysis in patients with acute ischemic stroke: a randomized trial.* Stroke 2014;**45**:159-67.
19. Sloth, A.D., et al., *Improved long-term clinical outcomes in patients with ST-elevation myocardial infarction undergoing remote ischaemic conditioning as an adjunct to primary percutaneous coronary intervention.* Eur Heart J 2014;**35**:168-75.

Summary of Changes

Protocol

Amendment, date	**Details**
1. Amendment, 04.02.2015	Adding the assessment of N-terminal pro-Brain Natriuretic Peptide (Zealand University Hospital trial site)
2. Amendment, 04.06.2015	Adding a trial site (Regional Hospital West Jutland) At the new trial site, high-sensitive troponin I was assessed. Myocardial injury was defined as high-sensitive troponin I > 24 ng/L (99_{th} percentile URL, 14% CV)
3. Amendment, 13.08.2015	Adding a trial site (Herlev Hospital) Excluding patients undergoing major abdominal surgery Adding an assessment of endothelial function and coagulation markers
4. Amendment, 25.01.2016	Adding the assessment of N-terminal pro-Brain Natriuretic Peptide (Regional Hospital West Jutland trial site)
5. Amendment, 29.12.2016	Adding the exclusion criteria: "Other conditions that prevent the performance of remote ischemic preconditioning" Expanding the clinical follow-up until 5 years after surgery

Original version

Prevention of myocardial injury by remote ischemic preconditioning in emergent or urgent non-cardiac surgery: a randomized clinical trial

The PIXIE study

Statistical analysis plan for the primary publication

Definitions of the population

Intention-to-treat: randomized in the trial and a minimum of two postoperative troponin assessments within four days of surgery (an evaluable primary outcome)

Per-protocol: randomized in the trial, received the allocated treatment, and a minimum of two postoperative troponin assessments within four days of surgery

We will do a per-protocol analysis and an intention-to-treat analysis.

The primary analysis

Comparing the incidence of myocardial injury (No. (%)) in the intervention group versus the control group. We plan to use a chi-square test. The difference is considered to be significant if the two-sided p-value < 0.05.

Secondary analyses

All analyses compare the difference between the intervention group and the control group. Continuous outcomes will be analyzed with non-parametric (Wilcoxon two-sample test) or parametric statistics (unpaired t-test) depending on data distribution and equality of variances. If necessary data will be logarithm transformed. Continuous outcomes will be expressed as mean (95% confidence interval) or median (inter-quartile range). Categorical outcomes will be analyzed with a chi-square test or Fischer's exact test (an expected count < five in one cell) and the result will be expressed as no. (%). Differences are considered significant if the two-sided p-value < 0.05.

Secondary comparisons include

- Peak plasma troponin I within four days of surgery
- Total troponin I release (area under the curve) during the first four days after surgery
- Perioperative myocardial infarction

Original version

- Major adverse cardiovascular events (30 days)
- Length of postoperative hospital stay
- Length of intensive care unit stay
- All-cause mortality (30 days)

Sample size

We predict that 15% of the patients in the placebo group will suffer from myocardial injury and that the incidence of myocardial injury will be reduced to 7% in the intervention group. Type I error is set at 5% and type II error is set at 20%. In total 2 x 264 patients need to be included based on this power calculation. We will include patients until we have a total of 2 x 270 patients for evaluation (per-protocol).

We will do an interim analysis based on the primary outcome when a total of 270 (50%) evaluable patients have been included in the study. We will use the O'Brien Fleming method with a level of significance at $p<0.005$ (first analysis) and $p<0.048$ (second analysis).

Final version

Prevention of myocardial injury by remote ischemic preconditioning in emergent or urgent non-cardiac surgery: a randomized clinical trial

The PIXIE study

Statistical analysis plan for the primary publication

Definitions of the population

Intention-to-treat: randomized in the trial and a minimum of two postoperative troponin assessments within four days of surgery (an evaluable primary outcome)

Per-protocol: randomized in the trial, received the allocated treatment, and a minimum of two postoperative troponin assessments within four days of surgery

We will do a per-protocol analysis and an intention-to-treat analysis.

The primary analysis

Comparing the incidence of myocardial injury (No. (%)) in the intervention group versus the control group. We plan to use a chi-square test. The difference is considered to be significant if the two-sided p-value < 0.05.

Secondary analyses

All analyses compare the difference between the intervention group and the control group. Continuous outcomes will be analyzed with non-parametric (Wilcoxon two-sample test) or parametric statistics (unpaired t-test) depending on data distribution and equality of variances. If necessary data will be logarithm transformed. Continuous outcomes will be expressed as mean (95% confidence interval) or median (inter-quartile range). Categorical outcomes will be analyzed with a chi-square test or Fischer's exact test (an expected count < five in one cell) and the result will be expressed as no. (%). Differences are considered significant if the two-sided p-value < 0.05.

Secondary comparisons include

- Peak plasma troponin I within four days of surgery (stratified on cardiac troponin I and high-sensitive troponin I)

- Total troponin I release (area under the curve) during the first four days after surgery (stratified on cardiac troponin I and high-sensitive troponin I)
- Perioperative myocardial infarction
- Major adverse cardiovascular events (30 days)
- Length of postoperative hospital stay
- Intensive care unit admission
- All-cause mortality (30 days)

To analyze the association between the intervention and the primary outcome, myocardial injury, we will do a univariate and multivariate logistic regression. The results will be expressed as odds ratios (95% confidence interval).

The decision to include a variable in the multivariate logistic regression will be based on clinical hypotheses. Trial site effect will be analyzed. A maximum of one variable per 10 primary events will be included.

Sample size

We predict that 15% of the patients in the placebo group will suffer from myocardial injury and that the incidence of myocardial injury will be reduced to 7% in the intervention group. Type I error is set at 5% and type II error is set at 20%. In total 2 x 264 patients need to be included based on this power calculation. We will include patients until we have a total of 2 x 270 patients for evaluation (per-protocol).

Summary of Changes

Statistical Analysis Plan

Amendment, date	**Details**
1. Amendment, 04.06.2015	Peak plasma troponin I and total troponin I release (area under the curve): The analyses will be stratified on cardiac troponin I and high-sensitive troponin I
2. Amendment, 25.01.2016	We decided not to perform the interim analysis. No data analysis had been performed and the investigators were blinded.
3. Amendment, 01.09.2017	The addition of a univariate and multivariate logistic regression to analyze the association between the intervention and the primary outcome. The analyses were added before the study inclusion was terminated. No data analysis had been performed and the investigators were blinded.